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REVIEW

Interactions of transgenic *Bacillus thuringiensis* insecticidal crops with spiders (Araneae)

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Abstract. Genetically modified crops expressing insecticidal proteins from *Bacillus thuringiensis* (Bt) have dramatically increased in acreage since their introduction in the mid-1990's. Although the insecticidal mechanisms of Bt target specific pests, concerns persist regarding direct and indirect effects on non-target organisms. In the field, spiders may be exposed to Bt toxins via multiple routes, including phytophagy and pollenivory, consumption of Bt-containing prey, and soil exudates in the detrital food web. Beyond direct toxicity, Bt crops may also have indirect impacts, including pleiotropic and prey-mediated effects. Here, we comprehensively review the literature and use meta-analyses to reveal that foliar spider abundance is unaffected by Bt corn and eggplant, while cotton and rice revealed minor negative effects and there were positive effects from potato. Moreover, the soil-dwelling community of spiders was unaffected by Bt corn and cotton, while positively impacted in potato. However, Bt crops had higher populations of both foliar and epigeal spiders than insecticide-treated non-Bt crops. The current risk-assessment literature has several caveats that could limit interpretations of the data, including lack of taxonomic resolution and sampling methods that bias the results in favor of certain spiders. These families responded differently to Bt crops, and spider responses to insecticides are species- and toxin-specific, thus highlighting the need for greater taxonomic resolution. Bt crops have become a prominent, and increasingly dominant, part of the agricultural landscape; understanding their interactions with spiders, a diverse and integral component of agroecosystems, is therefore essential.

Keywords: Spiders, genetically modified organisms, GMO, non-target risk-assessment, agroecosystem, Bt toxin

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1. INTRODUCTION

The adoption of biotechnology in agriculture has been employed in the global push toward sustainable intensification of crop productivity, in an attempt to meet demands for increased food security for a growing worldwide population. The planting of genetically modified crops has been widespread; in 2009, 135 million hectares of biotech crops were grown by an estimated 14 million farmers in 25 countries (James 2009). Insect-resistant genetically modified crops (e.g., *Bacillus thuringiensis* [Bt] crops) have become dominant fixtures in many of the world's agricultural regions (James 2007; Naranjo 2009). The replacement of conventional crops with their Bt counterparts is thereby altering the composition and dynamics of agroecosystems across regional and global scales.

Bt crops are genetically engineered to express insecticidal proteins of the entomopathogen *Bacillus thuringiensis* Berliner 1915 (Bacillales: Bacillaceae). Transgenic plants are modified by inserting a gene from *B. thuringiensis* into the genome of the crop plant, termed a transgenic event, thereby allowing the crop to express insecticidal proteins in its own tissues. The insecticidal proteins expressed in these transgenic crops are known as Bt δ-endotoxins/Cry proteins. The insecticidal mode of action occurs when Bt toxins are ingested by insect pests; these proteins bind to receptors on the midgut lining of susceptible individuals, causing lysis of epithelial cells on the gut wall and perforations in the midgut lining, which stops feeding and causes death by septicemia (Glare & O'Callaghan 2000). Bt toxins target a fairly narrow spectrum of pest insects that possess specific physiological traits (i.e., gut pH and toxin receptor sites in the midgut) and thus intuitively pose less risk to non-target species than broad-spectrum insecticides (Marvier et al. 2007; Wolfenbarger et al. 2008; Naranjo 2009; Duan et al. 2010). For example, Cry1 proteins are effective against certain lepidopterans, and Cry3 proteins affect certain coleopterans. Despite the relative safety in comparison to conventional insecticides and economic benefits to growers (Hutchinson et al. 2010), there is still concern that Bt crops could have non-neutral interactions with non-target organisms, such as spiders.

Current risk-assessment literature has focused on the direct and indirect effects of transgenic Bt crops on a variety of non-target taxa, including important arthropod predator groups such as ladybird beetles (Coleoptera: Coccinellidae) (e.g., Lundgren & Wiedenmann 2002; Harwood et al. 2007), ground beetles (Coleoptera: Carabidae) (e.g., French et al. 2004; Zwahlen & Andow 2005; Duan et al. 2006; Harwood et al. 2006; Peterson et al. 2009), lacewings (Neuroptera: Chrysopidae) (e.g., Hilbeck et al. 1998; Dutton et al. 2002; Guo et al. 2008), and true bugs (Hemiptera) (e.g., Al-Deeb et al. 2001a; González-Zamora et al. 2007; Duan et al. 2007). Within the arachnids, non-target studies have focused primarily on predatory mites (Acari: Phytoseiidae), and the majority of these studies have found no negative impacts of Bt toxins (e.g., Obrist et al. 2006a; Esteves et al. 2010). In contrast to the abundant risk-assessment literature addressing predatory mites, spiders have received a particularly low level of attention in proportion to their importance in cropping systems.

Therefore, this review will address the interactions between Bt crops and spiders in transgenic agroecosystems, forming a framework for risk-assessment by reviewing the role of spiders in agroecosystems and the direct and indirect routes by which Bt crops may affect spider communities. Subsequently, literature examining the consequences of this exposure to Bt toxins for spider fitness and fecundity is reviewed. Additionally, the effects of Bt crops at the community level, as measured by abundance of foliar and soil-dwelling spiders in the field, are evaluated using meta-analysis to examine both crop- and family-specific effects. A discussion of the literature reviewed will address limitations of these studies and implications of spider responses to chemical insecticides for Bt crop risk-assessment. This review provides a synthesis of field- and laboratory-based studies of the impacts of *Bacillus thuringiensis* crops on the diverse and agriculturally significant spider community.

2. ROLE OF SPIDERS IN AGROECOSYSTEMS

As generalist predators, spiders have often been overlooked in the context of biological control of insects (DeBach & Rosen 1991; Hoy 1994), despite their ubiquitous nature and high abundance in agricultural fields (Riechert & Lockley 1984). However, generalist predator species assemblages can significantly reduce pest populations in many cases (reviewed by Symondson et al. 2002). Polyphagous habits may allow some predators to survive the high levels of disturbance in agricultural settings (Murdoch et al. 1985), meaning that generalists are often the principal predators in annual crops.

2.1 Prevalence of spiders in croplands.—Indeed, spiders often dominate the agroecosystem, in part due to their ability to reach high population densities. Nyffeler & Sunderland (2003) reported 2–600 spiders per m^2 in European field crops, consisting primarily of linyphiids, while only 0.02–14 spiders per m^2 were found in North American annual crops. However, recent studies have found higher population densities in the USA: 19 spiders per m^2 on the soil surface in annual field crops in Illinois, (Lundgren et al. 2006) and an average of 67 spiders per m^2 on the soil surface in early season field corn in South Dakota, (Lundgren & Fergen, in press). Spider communities in agroecosystems can also be very diverse; over 600 combined species of spiders were found across nine field crops in U.S. agriculture (Young & Edwards 1990). Spiders represent a major portion of the invertebrate predators found in terrestrial ecosystems, and their populations often outnumber other predatory arthropods in a diversity of habitats.

2.2 Biological control potential.—Spiders are capable of capturing a significant proportion of the insects in the trophic level below them, as well as at their own trophic level (Wise 1993). For example, spiders are responsible through direct predation and non-consumptive effects for a reduction of up to 42% of pest cutworm larvae in tobacco (Nakasuji et al. 1973) and 49% of pest aphid populations in cereal crops in the United Kingdom (Chambers & Aikman 1988). Thus, spiders, in conjunction with other natural enemies present within agroecosystems, can exert a positive synergistic effect on pest population dynamics (Sunderland et al. 1997). Additionally, spiders are more likely to remain in agroecosystems during periods of low prey abundance than to disperse to surrounding areas (Greenstone 1999), allowing for greater predation on prey species once they enter a cropping system. Spiders also exert synergistic biological control effects via partial consumption of caught prey (Haynes & Sisojevic 1966; Samu 1993) or without consumption by dislodging pests from plant surfaces (Nakasuji et al. 1973; Mansour et al. 1981), causing mortality in webs (Nentwig 1987; Alderweireldt 1994), altering pest behavior via predation risk (Schmitz et al. 1997) and "superfluous killing" (Provencher & Coderre 1987; De Keer & Maelfait 1988; Mansour & Heimbach 1993; Samu & Biro 1993; Maupin & Riechert 2001) (reviewed by Sunderland 1999). Linyphiidae in particular are known to build their webs selectively at micro-sites with high prey density and diversity (Harwood et al. 2001, 2003; Harwood & Obrycki 2007; Romero & Harwood 2010). Agrobiont spider species (reaching high dominance in agroecosystems [Samu & Szinetár 2002]), display a number of life history traits allowing them to persist in annual agroecosystems despite frequent disturbances and periods of prey scarcity, including high egg production, an extended breeding season, multiple generations per year, the ability to immigrate into annual crops early in the season via ballooning, and low metabolic rates (Anderson 1970, 1996; Greenstone & Bennett 1980; Anderson & Prestwich 1982; Bishop & Riechert 1990; Nyffeler & Breene 1990; Schmidt & Tscharntke 2005). These life history traits make linyphiids important biological control agents and a major component of ecological webs in agroecosystems (Thorbek et al. 2004).

Spiders may also contribute to biological control efforts if these generalist predators are able to move into a cropping system early in the season (Sunderland et al. 1997). The ballooning ability of spiders, particularly Linyphiidae, which can exhibit this behavior at both immature and adult stages (Weyman et al. 1995), allows these predators to rapidly colonize a cropping system following cultivation of the field (Riechert & Lockley 1984; Sunderland et al. 1986). Spiders can then build their populations by subsisting on alternative non-pest prey or non-prey resources before pests arrive; this 'lying in wait' strategy may allow the predators to exert significant control over the pest population and even drive it to extinction (Murdoch et al. 1985). For example, in winter wheat in the United Kingdom, Collembola are an abundant alternative prey resource for linyphiid spiders early in the growing season (Harwood et al. 2003); the presence of this alternative food resource maintained spiders in the field and allowed for greater predation rates on pest aphids when their populations increased later in the growing season (Harwood et al. 2004). Similarly, Settle et al. (1996) found populations of generalist predators in rice were supported early in the season by detritivorous alternative prey.

2.3 Importance of diverse spider assemblages.—Although individual spider species do not exert significant biological control on agricultural pests, the multi-species spider assemblages found in agroecosystems can provide valuable suppression of pest populations (Greenstone 1999). Spider assemblages can cause mortality of nearly all life stages of an agricultural pest due to their variation in foraging behavior, diel activity, microhabitat selection, and size across species. Spiders found within agroecosystems occupy a wide range of ecological niches, which often leads to the grouping of spiders displaying similar foraging behaviors into guilds (Uetz 1977; Post & Riechert 1977; Uetz et al. 1999). However, within these guilds finer taxonomic resolution may yield differences in prey resource utilization (e.g., the subfamilies Erigoninae and Linyphiinae [Harwood et al. 2003]).

3. ROUTES TO EXPOSURE

Bt crops may affect non-target species residing within higher trophic tiers in two ways: via direct effects of the toxin following ingestion and/or via changes to the structure of agroecosystems that are associated with the widespread adoption of Bt crops (Lundgren et al. 2009a). Depending on the gene promoter that is used in a particular transgenic event and crop, the insecticide's final distribution and concentration within the plant may include any of a variety of tissues and exudates, including root and vegetative tissue, flowers, nectar, or pollen (Shi et al. 1994; Hilder et al. 1995; Rao et al. 1998; Couty et al. 2001; Raps et al. 2001; Bernal et al. 2002a; Wang et al. 2005; Wu et al. 2006; Burgio et al. 2007). Combined with their diversity and generalist feeding habits, routes to exposure are potentially complex for spiders (Fig. 1).

3.1 Consumption of pollen.—Bt proteins are often present in crop pollen and other plant tissues. Feeding directly on pollen, or on silk that has intercepted pollen, present direct routes of exposure to Bt toxins. Concentration of insecticidal Bt proteins in pollen varies depending on the crop type, transgenic event, and phenology, as well as factors of the region and environment (Fearing et al. 1997; Duan et al. 2002; Grossi-de-Sa et al. 2006; Obrist et al. 2006b). Pollen is a component of the diets of some generalist predators, including spiders; a pollen-based diet can increase spiderling survival of select groups, including a crab spider *Thomisus onustus* Walckenaer 1805 (Thomisidae) (Vogelei & Greissl 1989) and an orb-web spider *Araneus diadematus* Clerck 1757 (Araneidae) (Smith & Mommsen 1984). Orb-web spinning spiders located inside or around the borders of transgenic cornfields could also potentially consume Bt proteins from pollen blown by wind onto their webs. Despite its large size and typically rapid settling rate, corn pollen may travel up to 30 m from its source (Raynor et al. 1972). Pollen deposition can reach high levels in cornfields and their margins: 1,400 grains/cm^2 on milkweed leaves (Pleasants et al. 2001) and over 200 grains/cm^2 in simulated linyphiid spider webs (Peterson et al. 2010). For spiders that re-ingest their webs in order to recycle the silk and rebuild their webs daily (e.g., some araneids), this behavior could facilitate the ingestion of pollen that dusted their webs during anthesis (Ludy 2004; Ludy & Lang 2006a). The sheet-web weaving spiders (Linyphiidae) readily consume

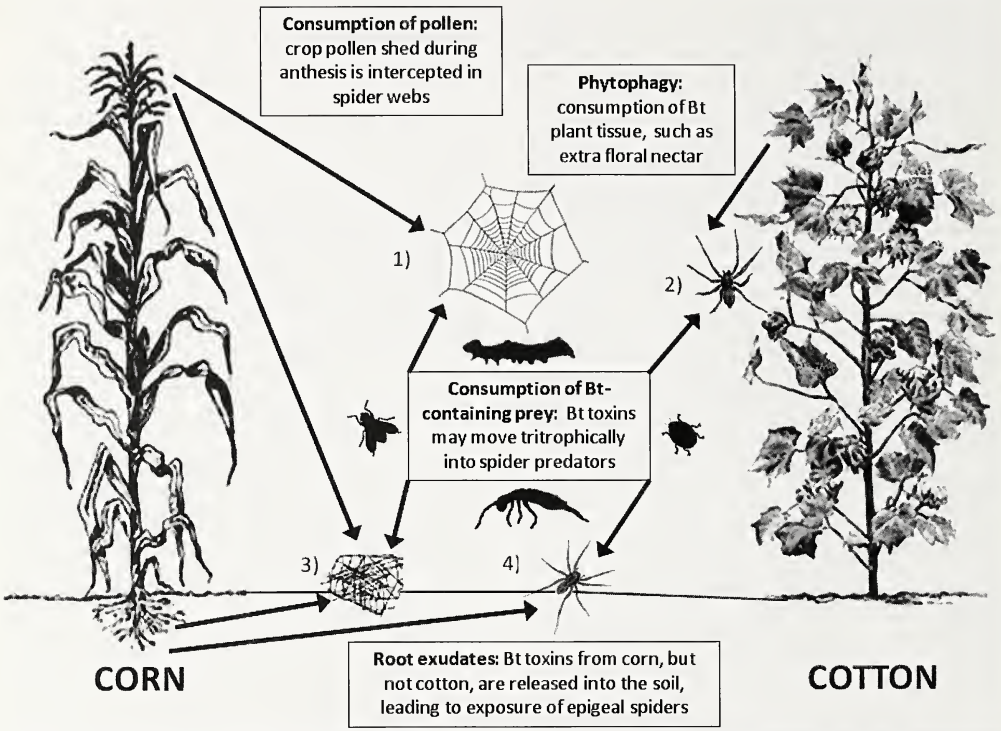

Figure 1.—Potential routes to Bt toxin exposure for spiders in transgenic agroecosystems. Sources and pathways for Bt toxin movement are highlighted for several spider families common in transgenic corn and cotton agroecosystems, including 1) Aranеidae, 2) Anyphaenidae, 3) Linyphiidae, and 4) Lycosidae.

pollen that has been intercepted in their webs (Sunderland et al. 1996; Peterson et al. 2010). The combination of high pollen deposition and low prey interception rates at ground-based linyphiid webs in transgenic corn maximizes the potential for pollen consumption and uptake of Bt toxins (Peterson et al. 2010). Thus, there is considerable exposure to pollen in many agroecosystems over a very short window of time (during anthesis), which may constitute a significant route to exposure to Bt toxins.

3.2 Other forms of phytophagy.—Many non-target species, including beneficial insects and spiders, rely on plant-based foods (reviewed by Wäckers 2005 and Lundgren 2009) and thus are at risk of being affected by Bt toxins, as toxin transfer can be facilitated by direct consumption of Bt-containing plant material (Dutton et al. 2002; Meissle et al. 2005; Obrist et al. 2005, 2006a, c). Despite the reportedly wide dietary breadth of spiders (Nyffeler et al. 1994), they are traditionally considered a strictly predaceous group. However, recent studies have shown the propensity of some spiders to utilize plant food resources, such as *Bagheera kiplingi* Peckham & Peckham 1896 (Salticidae) consuming the Beltian bodies of the acacia tree (Meehan et al. 2009) and several species of both the genus *Cheiracanthium* (Miturgidae) and *Hibana* (Anyphaenidae) consuming extra-floral nectar (Patt & Pfannenstiel 2008, 2009; Taylor & Pfannenstiel 2008, 2009; Taylor & Bradley 2009). Therefore, ingestion of plant material represents a potential pathway to Bt toxin exposure of non-target spiders in transgenic agroecosystems, although feeding frequency on plant resources (other than pollen) in transgenic crops has not been documented.

3.3 Consumption of Bt-containing herbivores or other prey.—Spiders may be exposed to Bt toxins through the consumption of prey that have fed on Bt tissue. Trophic linkages between spiders and prey can vary, based on the predator's foraging mode; aerial prey, such as Diptera, are of high importance to Tetragnathidae and less important to Lycosidae and Linyphiidae, while the opposite pattern of trophic strength is seen for Collembola, with this prey playing the largest role in the diet of linyphiids (Nyffeler & Sunderland 2003) and juvenile lycosids (Wise 1993; Oelbermann et al. 2008). Spiders in a transgenic agroecosystem are therefore likely to intercept and consume a potentially wide variety of prey, which may have been exposed to Bt toxins through their diet. Spiders are capable of consuming potentially Bt-containing prey items in

agricultural fields, such as seen in the trophic linkages between spiders and western corn rootworm *Diabrotica virgifera virgifera* LeConte (Coleoptera: Chrysomelidae) (Lundgren et al. 2009b). Additionally, secondary predation of smaller arthropod predators that contain Bt toxins may occur; some small, soft-bodied predatory insects, such as *Nabis roseipennis* Reuter 1872 (Hemiptera: Nabidae) and *Orius insidiosus* (Say 1832) (Hemiptera: Anthocoridae) show high uptake of Bt toxins in the field (Harwood et al. 2005) and could easily become prey to spiders.

3.4 Root exudates and the detrital food web.—Another potential route of transgenic protein movement to spiders is through the soil-based food web and ingestion of soil-dwelling arthropods via root exudates and plant biomass. Bt corn, potato, and rice all release transgenic protein-containing root exudates during plant growth; however, Bt canola, cotton, and tobacco do not (Saxena et al. 1999, 2004; Saxena & Stotzky 2000; Icoz & Stotzky 2007). Several studies have quantified the persistence of Bt toxins in the soil (Koskella & Stotzky 1997; Saxena et al. 2002; Zwahlen et al. 2003a; Stotzky 2004; Icoz & Stotzky 2008), with results indicating that Bt toxins will persist in the soil from 2–32 wk. This wide discrepancy in persistence times may be partially due to differences in microbial activity (Palm et al. 1996; Koskella & Stotzky 1997; Crecchio & Stotzky 1998), which is in turn affected by the pH and mineral content of soils (Icoz & Stotzky 2008). Bt toxins may bind to humic acids, organic supplements, or soil particles, protecting the toxins from degradation by microbes and extending the persistence of insecticidal activity in the soil (Glare & O'Callaghan 2000).

Exposure to Bt toxins via consumption of common soil-dwelling detritivores or herbivores by epigeal spiders common in agroecosystems (e.g., Lycosidae, Gnaphosidae, Linyphiidae) is likely due to their foraging habits. The presence of Bt toxins in the soil, as well as the consumption of fresh or decaying transgenic plant material, can lead to exposure of soil-dwelling organisms, such as Collembola, slugs, and earthworms (Zwahlen et al. 2003b). Collembola are readily consumed by spiders and represent a major trophic linkage; linyphiids will build their webs at micro-sites with high Collembola abundance (Harwood et al. 2001, 2003). Although spiders are capable of consuming earthworms (Nyffeler et al. 2001) and slugs (Nyffeler & Symondson 2001), these prey are not a major resource utilized by these generalist predators. Depending on the crop and agronomic aims of the grower, large amounts of crop residues may be churned into the soil during the harvesting process, allowing for further Bt toxin exposure in soil-dwelling communities, although this is not the case when all crop material is removed during harvest (e.g., corn destined for ethanol production [Giampietro et al. 1997]).

3.5 Indirect effects.—In addition to direct toxicity, the production of Bt toxins by Bt crops changes the agroecosystem relative to non-transgenic cropland in several ways that have important implications for food web dynamics. First, insertion of the gene complex into the crop plant may result in unpredicted and unintended pleiotropic effects changing the plant from its non-transgenic counterpart (Picard-Nizou et al. 1995; Saxena & Stotzky 2001; Birch et al. 2002; Faria et al. 2007). For example, a reported pleiotropic effect in Bt corn is an increase in the lignin content of transgenic plant tissue (Saxena & Stotzky 2001), which may lead to reduced decomposition in soil (Flores et al. 2005), although other studies have shown no differences in rate of decomposition for Bt tissue (Lehman et al. 2010; Zurbrugg et al. 2010). An additional pleiotropic effect of transformation in some transgenic corn may be an increase in attractiveness as an oviposition site for corn leafhoppers *Dalbulus maidis* (DeLong & Wolcott) (Hemiptera: Cicadellidae), a pest that is not targeted by Bt toxins, possibly due to altered plant traits that influence oviposition, such as leaf vein characteristics, foliar pubescence, or plant chemistry (Virla et al. 2010). Genetic transformation of potatoes can also decrease foliar expression of toxic glycoalkaloids (Birch et al. 2002). These altered plant characteristics may impact spiders, as variations at the plant level can have effects on higher trophic levels, including predators (Lundgren et al. 2009c; Pilorget et al. 2010). How pleiotropic effects impact spiders is poorly understood, although the potential consequences of these effects merit further research.

Perhaps more importantly, prey-mediated effects of Bt crops on higher trophic levels are well documented in the laboratory (Hilbeck et al. 1998; Bernal et al. 2002b; Dutton et al. 2002; Ponsard et al. 2002; Romeis et al. 2004, 2006; Lövei & Arpaia 2005; Hilbeck & Schmidt 2006; Torres & Ruberson 2006; Naranjo 2009), although studies addressing spiders have been neglected. This multitrophic-level effect occurs when the fitness or performance of target or non-target prey that consume Bt tissue is reduced. As a result, prey may be of lesser quality or reduced abundance in Bt fields, and thus a bottom-up effect may be triggered that could affect the foraging or fitness of higher trophic levels, such as spiders (but see Torres & Ruberson 2008). Moreover, reduced prey availability may increase the likelihood that generalist predators will directly consume Bt toxins by feeding on plant-provided resources to supplement their diet (e.g., Al-Deeb et al. 2001b). Any non-neutral effects of Bt crops on spiders, whether direct or indirect, could have implications for biological control and food-web structure.

4. UPTAKE OF BT TOXINS BY SPIDERS

Despite their potential to play an important role in biological control programs and the multitude of pathways through which spiders may be exposed to Bt toxins in agroecosystems, few studies have addressed the uptake of Bt toxins in the field, as well as consequences of such exposure to spiders. Key components of non-target risk-assessment are determining the level of exposure and harm of Bt toxins, and studies involving spiders are essential.

4.1 Evidence for Bt toxin uptake by spiders in the field.—Studies documenting the presence or absence of transgenic proteins in the gut contents of spiders are scarce. Harwood et al. (2005) reported 7.7% of 91 field-collected spiders (dominated by Linyphiidae and Tetragnathidae) tested positive for Cry1Ab in field corn, indicating that exposure pathways exist for these spiders in transgenic corn. This is likely the only study in which field populations of spiders were screened for Bt toxins in a transgenic agroecosystem. Several generalist predators are better studied than spiders and regularly take up Cry1Ab in the field. These predators include ladybird beetles

(Coleoptera: Coccinellidae), ground beetles (Coleoptera: Carabidae), and damsel bugs (Hemiptera: Nabidae) (Zwahlen & Andow 2005; Obrist et al. 2006b; Harwood et al. 2005, 2007; Wei et al. 2008; Peterson et al. 2009).

4.2 Potential consequences of consuming Bt toxin.—Laboratory studies of the movement of Bt toxins through spider-based food webs, as well as the consequences of consuming these transgenic proteins on the fitness and fecundity of spider predators, are also scarce. Lövei & Arpaia (2005) point out the lack of laboratory studies using spiders, as well as several other arthropod groups, as a "striking omission" in the Bt risk-assessment literature.

Laboratory-based feeding studies examining effects of Bt toxin on spiders via consumption of non-prey resources include an orb-weaver *A. diadematus*, which showed no change in survival, weight gain, reaction time, molt frequency, or web-building when juveniles were fed Cry1Ab corn pollen via web re-ingestion (Ludy & Lang 2006a). Similarly, adults and juveniles of a tangle-web spider *Phylloneta impressa* (L. Koch 1881) (Theridiidae) fed Cry3Bb1-containing prey or pollen for eight weeks had no effect on mortality, weight gain, development, or fecundity (Meissle & Romeis 2009).

Additional studies have examined the tritrophic movement of Bt toxins into spiders via their herbivorous prey. Jiang et al. (2004) fed transgenic rice expressing Cry1Ab Bt toxins to two herbivorous insects: the striped stem borer *Chilo suppressalis* (Walker 1863) (Lepidoptera: Crambidae) and the Chinese brushbrown caterpillar *Mycalesis gotama* Moore 1857 (Lepidoptera: Nymphalidae). These prey were subsequently fed to a wolf spider, *Pirata subpiraticus* (Bösenberg & Strand 1906) (Lycosidae). Antibody assays of each trophic level indicated Bt toxins were transferred up the food chain from transgenic rice to both prey species and into the spider; however, Cry1Ab concentration diminished with each step up the food chain, and the two prey species transferred Cry1Ab up the food chain with different efficiencies (Jiang et al. 2004). Similarly, Chen et al. (2009) tracked the movement of Cry1Ab from Bt rice into *P. subpiraticus* via a leaffolder *Cnaphalocrocis medinalis* (Lepidoptera: Pyralidae). In addition to showing that Cry1Ab concentration decreased as it moved through the food chain (herbivores contained approximately 0.6–1.1 Cry1Ab/fresh weight [μg/g] and predators contained 0.06–0.12 [μg/g]), this study also demonstrated a lack of binding of Cry1Ab molecules to the mid-gut lining of *P. subpiraticus*. Although fecundity and survivorship measures were unaffected, development time was significantly longer for spiders consuming Cry1Ab-containing prey, potentially due to indirect effects of reduced prey quality (Chen et al. 2009). Delayed development could have important consequences in the field, potentially increasing predation risk, including cannibalism and intraguild predation, which can have strong impacts on wolf spider populations (Wagner & Wise 1996; Hodge 1999). In a similar study system, Tian et al. (2010) examined the tritrophic movement of Cry1Ab from rice to herbivorous brown planthoppers *Nilaparvata lugens* (Hemiptera: Delphacidae) and their spider predators, *Ummeliata insecticeps* (Bösenberg & Strand 1906) (Linyphiidae). Cry1Ab concentration decreased as trophic level increased, with the planthopper-linyphiid uptake pathway demonstrating lower Cry1Ab mean concentrations (0.010 and 0.002 Cry1Ab/fresh weight [μg/g], respectively) (Tian et al. 2010) than the leaffolder-wolf spider pathway (Chen et al. 2009). These differences highlight the impact prey choice can have on a spider's likelihood for Bt toxin uptake in the field. Under current commercialized Bt toxin expression systems, phloem-feeders, such as brown planthoppers are less likely to take up Bt toxins than chewing insects, such as leaffolders, and therefore may convey lower concentrations of transgenic proteins to spiders (Raps et al. 2001).

5. EFFECTS OF BT CROPS ON SPIDER ABUNDANCE AND DIVERSITY

Risk-assessment research addressing the impacts of transgenic technology on spider populations has been published for six of the most common Bt crops. These studies varied widely in many research parameters, including type of Bt toxins expressed, region where fieldwork was conducted, duration of study, sampling methods, and outcomes (Table 1).

5.1 Meta-analysis.—Meta-analyses can reveal cross-study trends in the effects of Bt crops against non-target species that are not readily apparent from examining the results of individual studies, so we used this technique to examine the effects of specific Bt crops on spider communities. Specific hypotheses tested were 1) do non-Bt crops (corn, potato, cotton, eggplant, and rice) have similar spider abundances relative to Bt-crops in the absence of insecticide use, and 2) do non-Bt crops (corn, potato, cotton) that have been treated with insecticides to manage insect pests have similar spider abundance relative to Bt crops? To address this question, we updated a database originally published by Wolfenbarger et al. (2008), which was derived from Marvier et al. (2007). Specific studies included in the current database are indicated in Table 1. The spider community was divided depending on sampling method; spiders collected with pitfall traps were distinguished from those collected with beat cloths, suction, sticky cards, whole plant counts and pan traps. The meta-analyses used Hedges' *d* as its effect size estimator (Hedges & Olkin 1985), with relative effect sizes assigned to each study based on the sample sizes, means and standard deviations of the two treatments compared. Contrasts between treatments were conducted such that a positive effect size represents a beneficial effect of the Bt crops over the non-Bt crops. Comparisons were made using MetaWin 2.1, and mean ± non-parametric bias-corrected bootstrap confidence intervals (representing 95% confidence limits) were calculated (Rosenberg et al. 2000). If the error intervals encompassed zero, the effect size was not considered to be significant. Small, medium, and large effect sizes were considered to be approximately 0.2, 0.4, and 0.6, respectively (Cohen 1988). The results of these meta-analyses are presented in Figures 2 and 3, and are discussed below.

5.2 Field corn.—Transgenic corn is the most abundant and widespread Bt crop; approximately 41 million hectares of genetically modified corn were planted worldwide in 2009 (James 2009) and 63% of all corn planted in the United States in 2010 contained at least one Bt gene (USDA NASS 2010a). Bt corn lines may express Cry1 or Cry2 Bt-endotoxins that target lepidopteran pests (primarily European corn borer *Ostrinia nubilalis* Hübner and Southwestern corn borer *Diatraea grandiosella* Dyar [Lepidoptera: Pyralidae]) and/or

Cry3 Bt-endotoxins that target coleopteran pests (corn rootworm *Diabrotica* spp. (Coleoptera: Chrysomelidae)). Due to the widespread planting of this crop, more field studies examining the impact of Bt field corn on spider abundance have been published than for any other crop.

Our meta-analyses have revealed that spider abundances are unaffected by Bt corn relative to non-Bt corn, provided that insecticides are not applied to the non-Bt fields (Fig. 2). Therefore, the planting of Bt corn as an alternative to insecticide applications may benefit spider populations. However, insecticides to control Bt-targeting pests were not applied universally prior to the adoption of Bt crops, due to annual variation in pest populations, cost of scouting for pests, and effectiveness of crop rotation in some growing areas (Smith et al. 2004). Insecticides targeting the European corn borer were applied to 7% of corn grown in the USA in 1997 (Shelton et al. 2002), and 25% of corn acreage was treated for corn rootworms in 2001 (USDA ERS 2010). For lepidopteran-targeting Cry1Ab corn, no differences in spider abundance (Pilcher et al. 1997; Lozzia & Rigamonti 1998; Lozzia et al. 1998; Lozzia 1999; Jasinski et al. 2003; Delrio et al. 2004; Daly & Buntin 2005; de la Poza et al. 2005; Eckert et al. 2006; Fernandes et al. 2007) or diversity (Volkmar & Freier 2003; Sehnal et al. 2004; Meissle & Lang 2005; Farinós et al. 2008) were found between Bt and non-Bt corn untreated with conventional insecticides, using a variety of sampling methods. Similarly, Cry3Bb1 corn had no effect on spider abundance in the absence of insecticides (Bhatti et al. 2002, 2005a; Al-Deeb & Wilde 2003). When untreated Bt corn and non-Bt plots treated with conventional insecticide applications are compared, many studies indicate significantly lower population abundance of spiders immediately following insecticide applications and season-long in the chemically treated fields than in both Cry1Ab (Dively 2005; Meissle & Lang 2005; Bruck et al. 2006) and Cry3Bb1 corn (Bhatti et al. 2002, 2005b). Seed treatments of neonicotinoids or foliar sprays of pyrethroid insecticides on both Bt and non-Bt corn also reduced spiders caught in pitfall traps (Ahmad et al. 2005). Reports of significant differences among spider populations in Bt versus non-Bt corn have often lacked consistency across growing seasons. One field study conducted in Germany reported significantly fewer spiders in Cry1Ab corn in one of the three years of the study, while there was no difference the remaining two years (Lang et al. 2005).

Determining the effect of Bt corn on individual spider species may reveal differences unseen at lower taxonomic resolution. For example, Toschki et al. (2007) reported increased activity-density of two spiders (*Bathyphantes gracilis* [Blackwall 1841] and *Tenuiphantes tenuis* [Blackwall 1852] [Linyphiidae]) and decreased activity-density in one species (*Meioneta rurestris* [C.L. Koch 1836] [Linyphiidae]) in Bt versus non-Bt corn. However, Cry1Ab corn had no effect on populations of *Oedothorax* (Linyphiidae), *Alopecosa* (Lycosidae), various tetragnathids, and juvenile linyphiids and lycosids (Candolfi et al. 2004).

When examined at the guild level, spiders grouped as "hunting" or "web-building" showed no significant differences in abundance due to Cry1Ab corn in the Czech Republic; however, populations of the family Theridiidae increased over the three year study period in conventional fields, while decreasing in Bt treatments, a result credited to temporal fluctuations in the population dynamics of these spiders (Řezáč et al. 2006). In contrast to those findings, Ludy & Lang (2006b) found that in one of the three years of their study, foliage-dwelling spiders were more abundant in Bt corn and surrounding nettle margins than in conventional fields. The same study found no significant differences in spider abundance for the remaining field seasons, as well as no difference in species richness or guild distributions based on transgenic treatment.

5.3 Sweet corn.—Some sweet corn hybrids express Cry1Ab that targets several lepidopteran pests, including European corn borer *Ostrinia nubilalis* Hübner 1796 (Pyralidae), corn earworm *Helicoverpa zea* (Boddie 1850) (Noctuidae), and fall armyworm *Spodoptera frugiperda* Smith 1797 (Noctuidae). Acreages devoted to sweet corn are small compared to field corn (0.76% of corn acres planted in the USA in 2009) (USDA NASS 2010a, b). This crop differs from field corn in having a shorter maturation rate, which allows for Bt toxins to be expressed at high levels throughout the growing season (Rose & Dively 2007). Additionally, pollen production can be three to five times greater in sweet corn than in field corn (Goss 1968, Cottrell & Yeargan 1998; Peterson et al. 2010). Therefore, trophic transfer of Bt-endotoxins via pollen consumption may play an important role in sweet corn agroecosystems.

Over the course of two growing seasons, spider abundance in pitfall traps and visual counts in transgenic and non-transgenic sweet corn plots were similar, although lambda-cyhalothrin (pyrethroid) insecticides reduced spider abundances regardless of transgenic status (Dively & Rose 2002; Rose & Dively 2007). Another study in sweet corn used vacuum sampling to measure non-target arthropod abundance; although sample sizes were low, no significant differences in abundance of spiders between transgenic and non-transgenic plots were reported for early-, mid-, and late-season plantings (Hassell & Shepard 2002). Thus, initial literature indicates that Bt sweet corn does not adversely affect the non-target spider community.

5.4 Cotton.—Bt cotton is genetically engineered to express Cry1Ac, Cry1F, Cry2Ab and/or Vip3A proteins, which target lepidopteran pests in the bollworm complex (the genera *Helicoverpa* and *Heliothis* [Noctuidae], as well as *Pectinophora* [Gelechiidae]). Genetically altered cotton is widespread; approximately 14.5 million ha of Bt cotton was planted globally in 2009 (James 2009) and in the U.S., 73% of all cotton planted in 2010 contained the Bt gene (USDA NASS 2010a). Bt cotton has significantly reduced insecticide inputs in numerous cotton-growing regions of the world, including the United States (Betz et al. 2000; Gianessi & Carpenter 1999), China (Pray et al. 2001), and South Africa (Thirtle et al. 2003). The potential impact of Bt cotton on spiders could have implications for biological control. Spiders can be important predators of key lepidopteran pests of cotton (Mansour 1987) and have been capable of maintaining pests below the economic threshold (Breene et al. 1990). For example, cursorial spiders (Anyphaenidae and Miturgidae) consume eggs and larvae of the cotton bollworm *Helicoverpa zea* (Boddie 1850) (Lepidoptera: Noctuidae) (Renouard et al. 2004; Pfannenstiel 2008).

Table 1.—Summary of literature comparing abundance and/or diversity between Bt and non-Bt crops, listed by crop, Bt toxin/s expressed, geographic region, taxonomic resolution for statistical comparisons, and sampling method/s: 1. Pitfall trapping; 2. Yellow sticky cards in foliage; 3. Visual counts; 4. Destructive sampling of corn ears; 5. Vacuum-suction sampling; 6. Beat sheet/net/bucket collection; 7. Destructive sampling of whole plant; 8. Stem elector; 9. Emergence traps; 10. Pan trapping (modified Berlese of soil and roots); 11. Sweep-netting; 12. Drop cloth sampling. Asterisks indicate the studies providing data that could be used in the meta-analyses. [a] Only collecting methods in which spiders were caught are listed.

Crop	Bt toxin/s expressed	Geographic region		Taxonomic resolution	Sampling method/s[a]	References
Field corn	Cry1Ab	North America	Iowa, USA	Arachnida	1, 2, 3	Bruck et al. 2006*
					3	Pilcher et al. 1997*
			Georgia, USA	Araneae	1, 3	Daly & Buntin 2005*
			Ohio, USA	Araneae	2	Jasinski et al. 2003*
		Europe	Germany	Araneae	3	Lang et al. 2005*
					4	Eckert et al. 2006*
				Guild, family, genus or species	1	Volkmar & Freier 2003; Toschki et al. 2007
					5	Ludy & Lang 2006b*
					5, 6, 7, 8	Meissle & Lang 2005*
			Italy	Arachnida	2, 3	Delrio et al. 2004*
				Araneae	1, 3, 5	Lozzia & Rigamonti 1998; Lozzia et al. 1998; Lozzia 1999*
			Spain	Araneae	1, 3	de la Poza et al. 2005*
				Genus or species	1	Farinós et al. 2008*
			France	Family, genus or species	1, 6	Candolfi et al. 2004
			Hungary	Araneae	3	Árpás et al. 2005*
			Czech Republic	Guild, family or species	1	Řezáč et al. 2006*
					1, 7	Sehnal et al. 2004*
	Cry1Ab + Vip3A	North America	Maryland, USA	Araneae	1, 2, 3, 9	Dively 2005*
		South America	Brazil	Araneae	1, 2	Fernandes et al. 2007*
	Cry3Bb1	North America	Illinois, USA	Araneae	2	Bhatti et al. 2005a*
					1, 10	Bhatti et al. 2005b
					1, 2, 10	Bhatti et al. 2002*
			Kansas, USA	Araneae	1	Al-Deeb & Wilde 2003*; Ahmad et al. 2005*
Sweet Corn	Cry1Ab	North America	Maryland, USA	Araneae	1, 2, 3	Dively & Rose 2002*; Rose & Dively 2007
			South Carolina, USA	Araneae	5	Hassell & Shepard 2002
Cotton	Cry1Ac	North America	Arizona, USA	Araneae, family or species	7, 11	Naranjo 2005*
					7	Sisterson et al. 2004*
			South Carolina, USA	Araneae	6	Turnipseed & Sullivan 1999; Hagerty et al. 2000, 2005
			Georgia, USA	Araneae	1, 12	Torres & Ruberson 2005*
				Family, genus or species	1	Torres & Ruberson 2007*
			Tennessee, USA	Araneae	11	Van Tol & Lentz 1998
			Texas, USA	Araneae	6	Armstrong et al. 2000
			Alabama, Georgia & So. Carolina, USA	Araneae	6	Moar et al. 2002; Head et al. 2005*
		Asia	Henan, China	Araneae	3	Men et al. 2003, 2004*
				Species	3	Cui & Xia 1999
		Australia	New South Wales	Family	5	Whitehouse et al. 2005*
	Cry1Ab	Australia	New South Wales	Araneae	3	Fitt et al. 1994
	Cry1Ac + Cry2Ab	North America	Arizona, USA	Araneae, family or species	1, 11	Naranjo 2005*
			So. Carolina, USA	Araneae	6	Hagerty et al. 2005*
		Australia	New South Wales	Family	5	Whitehouse et al. 2005
	Cry1Ac + Cry1F	North America	New Mexico, USA	Family, genus or species	1, 6	Bundy et al. 2005*
	Cry1Ac + Cry1Ab	Asia	Hubei, China	Araneae	3	Deng et al. 2003
	Vip3A	Australia	New South Wales	Family	3, 5, 6	Whitehouse et al. 2007*

Table 1.—Continued.

Crop	Bt toxin/s expressed	Geographic region		Taxonomic resolution	Sampling method/s[a]	References
Potato	Cry3Aa	North America	Oregon, USA	Araneae	1	Duan et al. 2004*
					6	Reed et al. 2001*
			Maryland, USA	Araneae	1	Riddick et al. 2000*
		Europe	Sofia District, Bulgaria	Species	1	Kalushkov et al. 2008
Rice	Cry1Ab	Asia	Zhejiang, China	Araneae	5	Li et al. 2007
				Species	5	Chen et al. 2009*
	Cry1Ab + Cry1Ac	Asia	Zhejiang, China	Araneae	5	Li et al. 2007
				Family	5	Liu et al. 2003
				Species	5	Liu et al. 2002
Eggplant	Cry3Bb	Europe	Basilicata, Italy	Araneae	3	Arpaia et al. 2007*

Meta-analysis revealed a slight negative effect of Bt cotton on the abundance of foliar spiders relative to non-Bt fields, but this pattern was not seen in the soil spider community (Fig. 2). Bt cotton strongly supports spider abundance when compared to non-Bt cotton with insecticide applications, which simulates normal pest management practices (Fig. 2). Individual studies comparing Bt and non-Bt cotton fields untreated with insecticides reveal differing interpretations for abundances of foliar spiders (Fitt et al. 1994; Turnipseed & Sullivan 1999; Armstrong et al. 2000; Hagerty et al. 2000, 2005; Moar et al. 2002) and similar activity-densities of epigeal spiders (Torres & Ruberson 2007). When Bt cotton is compared with insecticide-treated conventional fields, spiders are more abundant in the Bt fields (Men et al. 2004; Head et al. 2005).

However, when spider populations are examined below the ordinal level, some differences between Bt and non-Bt cotton fields arise. Spider species from multiple families, including *Hylyphantes graminicola* (Sundevall 1830) (Linyphiidae) (Cui & Xia 1999), *Emblyna reticulata* (Gertsch & Ivie 1936) (Dictynidae) and *Mecaphesa celer* (Hentz 1847) (Thomisidae) (Naranjo 2005), showed no population differences in untreated Bt and non-Bt fields. Similarly, Salticidae (Naranjo 2005) and Clubionidae (Sisterson et al. 2004) were not affected by transgenic traits; however, in one study, the remaining spider community (lumped as "other Araneae") decreased in abundance in Bt cotton (Naranjo 2005).

5.5 Potato.—Transgenic potatoes express Cry3Aa targeting the Colorado potato beetle *Leptinotarsa decemlineata* Say 1824 (Coleoptera: Chrysomelidae), which is capable of decimating potato crops and costing farmers millions of dollars per year (Perlak et al. 1993; Kalushkov et al. 2008). Bt potatoes were grown commercially in the United States starting in 1995, but were withdrawn from the market in 2001 following pressure from anti-biotechnology groups and the lack of markets for Bt potato products (Kaniewski & Thomas 2004). However, this crop may see a resurgence in planting in Russia and eastern Europe in the near future (James 2009), as need for alternatives to costly insecticides for small-scale and subsistence farmers in these areas is great (Kaniewski & Thomas 2004). The spider community can dominate the epigeal predator fauna in potato fields (comprising up to 23% of total pitfall catches, second only to Collembola) (Duan et al. 2004), and may therefore play an important role in potato agroecosystems, highlighting the importance of assessing the impact of Bt potatoes on the spider community.

Although there were very few published studies on this topic, Bt potatoes tend to favor spider populations whether the non-Bt fields are sprayed with insecticides or not (Fig. 2). The adoption of Bt potatoes causes only a minor reduction in insecticidal applications, due to pest pressure from numerous species in addition to Bt-targeted Colorado potato beetles (Betz et al. 2000). As observed in previous crops, spraying non-Bt potatoes with insecticides has more of an impact on spider populations than Bt potatoes do (Riddick et al. 2000; Reed et al. 2001; Duan et al. 2004). However, Kalushkov et al. (2008) showed no significant differences in activity-density of spider species or community composition (measured by Sørensen similarity index) in response to insecticidal treatments or Bt potatoes. A similar meta-analysis to the one we ran on the abundance of non-target arthropods reported a positive effect of Bt potatoes on piercing/sucking insects, as well as generalist predators as a whole when compared to non-Bt potatoes (Cloutier et al. 2008). These authors believed that the increase in potential prey items was driving the increase in generalist predator populations.

5.6 Rice.—This crop has been engineered to express Cry1Ac and/or Cry1Ab for the control of several lepidopteran pests, including the striped stem borer *C. suppressalis* (Crambidae), yellow stem borer *Scirpophaga incertulas* (Walker 1863) (Pyralidae), and the leaffolder *Cnaphalocrocis medinalis* (Guenée 1854) (Pyralidae) (High et al. 2004; Wang & Johnston 2007). Although field trials with Bt rice have been conducted in China since 1998 (Tu et al. 2000), most transgenic lines are not yet commercially available. Agronomic practices in rice, such as periodic flooding of cultivated fields, shapes the insect community; in irrigated fields, up to 90% of arthropod diversity may be represented by freshwater species (Schoenly et al. 1998). Despite this, spiders have a long history of use in biological control programs in rice (e.g., Oraze et al. 1988; Heong et al. 1991; Sigsgaard 2007; Way & Heong 2009).

Our meta-analysis revealed a deleterious effect of Bt rice on spider abundance relative to non-Bt paddies (Fig. 2) (Chen et al. 2009). However, other field studies in China have found similar spider abundances in Bt and non-Bt rice paddies (Liu et al. 2002, 2003; Li et al. 2007). Additionally, Tian et al. (2010) focused on the population dynamics of the spider species *U. insecticeps* for three years in Bt and non-Bt rice

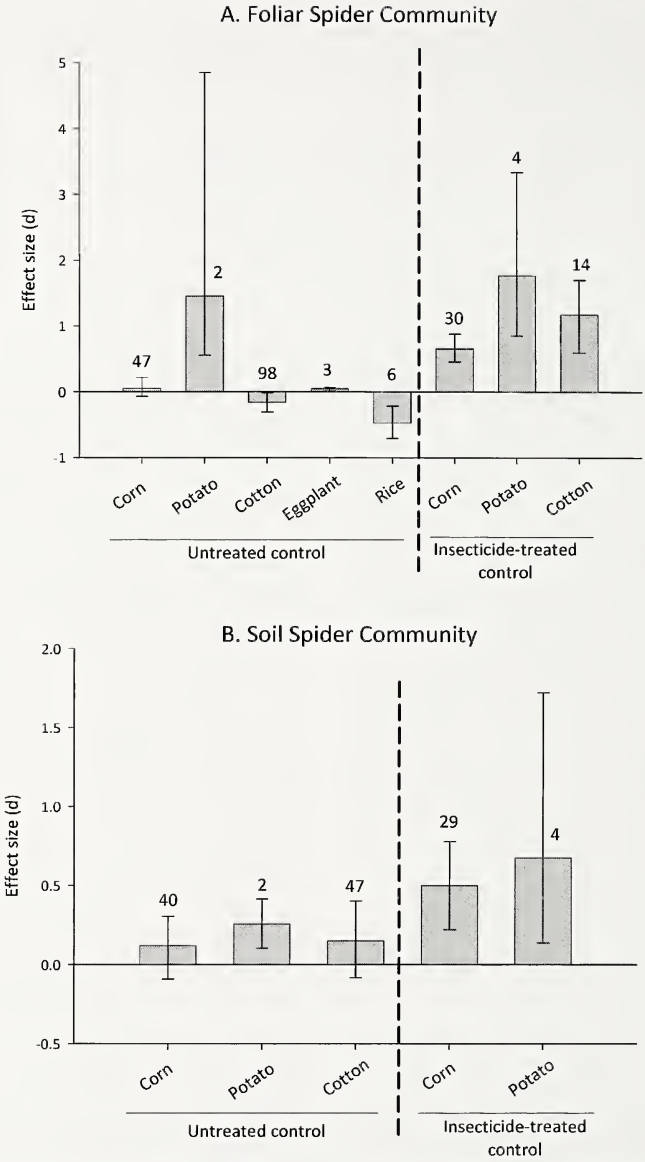

Figure 2.—The effects of Bt crops on foliar (A) and soil (B) communities of spiders, relative to insecticide-treated and untreated non-Bt controls. Positive bars indicate those crops in which spider abundance is favored by Bt treatment, and negative bars are crops in which spiders are less abundant in Bt-fields. Error lines represent biased 95% confidence intervals, and the numbers of observations for each system are noted above each bar.

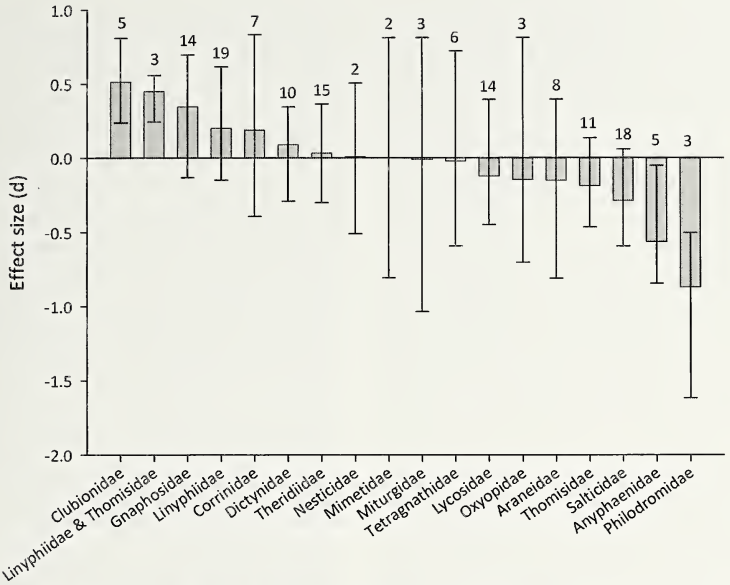

Figure 3.—The effects of Bt crops on spider families. Bars represent the effect sizes of Bt fields relative to non-Bt control fields that received no insecticides. Positive bars indicate those families favored by Bt treatment, and negative bars are families less abundant in Bt-fields. Error lines represent biased 95% confidence intervals, and the numbers of observations for each family are noted above each bar.

fields, reporting no differences for this predator; this linyphiid builds webs at the bottom of rice plants and is a major predator of the brown planthopper, *Nilaparvata lugens* (Stål 1854) (Hemiptera: Delphacidae) (Tian et al. 2010).

5.7 Eggplant.—Although the major contributors to Bt crop acreage worldwide are corn and cotton, other insect-resistant crops on the verge of commercialization, such as eggplant, could potentially see increased planting in the near future, particularly in India, where eggplant is a staple food (James 2009). Our meta-analysis revealed a slight, but significant positive effect of Bt eggplant over non-Bt eggplant (Fig 2). However, this analysis was based on a single study (Arpaia et al. 2007). Further research on the impact of Bt eggplant on spiders is necessary, particularly since the worldwide acreage of this crop may increase dramatically in the near future.

5.8 Other crops.—Additional Bt crops include oilseed rape (canola) (Stewart et al. 1996), tomato (Mandaokar et al. 2000), broccoli (Chen et al. 2008), collards (Cao et al. 2005), chickpea (Acharjee et al. 2010), spinach (Bao et al. 2009), soybean (Miklos et al. 2007), tobacco and cauliflower (Kuvshinov et al. 2001). However, these crops are not available commercially and are therefore very limited in their global planting. Despite some studies examining risk-assessment of these crops to non-target herbivores and natural enemies (e.g., Ferry et al. 2006; Chen et al. 2008; Romeis et al. 2009), no data exist for impact on spider populations in these transgenic agroecosystems.

5.9 Summary.—The spider risk-assessment literature is dominated by field studies conducted in the United States (48% of total references), Western Europe (23%), and China (15%). Studies in corn represent field sites in the U.S. and Europe, with just a single study from South America (Fernandes et al. 2007). Although Bt corn is grown in additional areas globally, such as Canada, South Africa, Egypt, and the Philippines (James 2009), these regions are not represented in the spider risk-assessment literature.

Overall, there was no consistent effect of Bt crops on spider abundance relative to non-Bt crops (Effect size = 0.01; 95% CIs ± 0.07; n = 268), but insecticides consistently have a greater negative effect on spiders than Bt crops do (Effect size = 0.73; 95% CIs ± 0.18; n = 81). However, a lack of taxonomic resolution, potentially biased methods of sampling, and a scarcity of studies in key geographic regions and crop types limits the completeness of the literature on this subject.

6. DISCUSSION

The existing risk-assessment literature allows some conclusions to be made on the effect of Bt crops on the spider community, which are predominantly non-negative. However, there are several limitations of these studies, including the lack of taxonomic resolution, use of collection techniques that may alter the perception of dominance within spider communities, and the variation in spider populations possibly due to crop type.

6.1 Interactions of Bt crops with spiders are often, but not always, neutral.—Bt crops can express one or multiple toxins that target a range of pests and are found in differing concentrations and distributions throughout the plant. This complexity, combined with the functional diversity of spiders and their often-intricate food webs, complicates the ability to make definite conclusions concerning the long-term effects of Bt crops on spiders. However, for the two most well-studied crops, corn and cotton, spiders appear to experience no direct negative effects from the adoption of Bt technology. Meta-analysis reveals no significant differences for total abundance of foliar and epigeal spiders when insecticides are absent, and spider abundance is more severely reduced when chemical applications are made than when Bt crops are planted without insecticides (Fig. 2). In contrast, the lesser-studied crops indicate non-neutral effects: Bt rice has fewer foliar spiders than non-Bt fields, while populations of soil and foliar spiders are greater in Bt potato (Fig. 2; but note the small number of observations in both of these systems). Also, some taxa within the Araneae (Anyphaenidae and Philodromidae) are adversely affected by Bt crops (Fig. 3).

The reasons for decreased spider abundance in rice and within certain taxa are not known, but it seems likely that these effects may be related to reductions in prey quality rather than direct toxicity of Bt proteins to spiders (Chen et al. 2009). Bt toxins are lethal to targeted pest species and cause the removal of those organisms from the agroecosystem; certain life stages of targeted pests are no longer available as potential prey items. Anyphaenids and philodromids are common in crops, such as cotton, where they are active foliar hunters most often collected by sweep-netting or beat sheet methods (Bundy et al. 2005). These families consume soft-bodied prey (Renouard et al. 2004; Pfannenstiel 2008), including Lepidoptera, which are targeted by the toxins expressed in Bt cotton. The absence of lepidopteran prey or their reduced quality due to feeding on Bt toxins may account for the observed negative effects of Bt crops on the families Anyphaenidae and Philodromidae (Fig. 3).

6.2 Greater taxonomic resolution is needed to reveal differential impacts of toxins on spiders.—Spiders are a diverse and abundant group within the predator community of Bt field crops (Duan et al. 2004; Sisterson et al. 2004; de la Poza et al. 2005). However, despite their prominent role, spiders have frequently been lumped into a single group at the order level for risk-assessment analysis (e.g., Fitt et al. 1994; Lozzia et al. 1998; Lozzia 1999; Turnipseed & Sullivan 1999; Armstrong et al. 2000; Reed et al. 2001; Bhatti et al. 2002, 2005a,b; Hassell & Shepard 2002; Deng et al. 2003; Duan et al. 2004; Ahmad et al. 2005; Daly & Buntin 2005; Eckert et al. 2006; Arpaia et al. 2007). The results of these studies are limited by their lack of taxonomic resolution. Spider communities occupy many functional niches, allowing for the ecological changes associated with Bt crops to affect spider species differentially. Studies of non-target impacts may reveal differences among treatments when data are examined in further taxonomic detail. For example, significant differences in the populations of several spider species in Bt vs. non-Bt crops were found when identified at greater taxonomic resolution (Naranjo 2005; Řezáč et al. 2006; Toschki et al. 2007).

Knowledge of the differential impact of insecticides on the abundance and fitness of spiders supports the hypothesis that Bt toxins will not affect spider species identically. For example, populations of a sheet weaver *Oedothorax apicatus* (Blackwall 1850) (Linyphiidae) responded negatively to applications of a pyrethroid insecticide, while a wolf spider (*Alopecosa* sp.) population was unaffected (Candolfi et al. 2004). Interactions of insecticides with spiders indicate both species- and insecticide-specific susceptibility, with frequent lethal (e.g., Fountain et al. 2007; Pekár & Beneš 2008) and sub-lethal effects (e.g., Deng et al. 2006; Tietjen & Cady 2007; Řezáč et al. 2010). Spider species also show differences in their susceptibility to certain chemical insecticides in the field; for example, populations of web-building spiders (Theridiidae) are less sensitive to certain types of insecticidal applications than ambush hunters (Philodromidae) (Bostanian et al. 1984). Susceptibility to insecticides is influenced by foraging mode, diel activity patterns, and web structure of spiders; one study found diurnal hunters and orb-web weavers were most susceptible to insecticides in the field (Pekár 1999). By extrapolating the results of the impact of other insecticidal products to the potential impact of transgenic Bt toxins on spiders, a pattern emerges. Individual spider species may be differentially affected, although it is important to note that Bt proteins are known to have a narrower range of toxicity than traditional insecticides.

We looked for patterns in the effects of Bt on different spider families, using a meta-analysis (using methods described above). The abundances of specific families in Bt versus non-Bt crops (without insecticides) vary substantially, suggesting that family-level effects of Bt crops are likely occurring but are being overlooked when spiders are grouped at the ordinal level (Fig. 3). These results highlight the need for specific study of spiders filling diverse and unique niches within an agroecosystem: large guild-level analyses grouping spiders into overly simplified groups may prevent any meaningful observation of treatment-level effects. It is therefore essential to study spiders in taxonomic detail, so that elucidation of potential differences among spider species is possible.

6.3 Collection techniques affect the perception of dominance within spider communities.—Sampling method strongly affects the number, diversity, and type of spiders collected (Amalin et al. 2001). Ecological traits of spider species, such as retreating behavior, can influence which collecting methods will be most effective. For example, wandering spiders using concealed retreats constructed from folded leaves and sticky silk (Anyphaenidae, Miturgidae) are easily observed visually, but are difficult to collect via methods such as vacuum-sampling or beat sheets that attempt to dislodge spiders from the habitat (Amalin et al. 2001). Therefore, the collecting method utilized by researchers in examining the spider communities in Bt versus non-Bt crops is likely to affect the results of these field studies.

Sampling methods varied widely within the non-target organism risk-assessment literature, although pitfall trapping was frequently used as a means to collect epigeal spiders and was often the only collection method utilized for spider capture (e.g., Riddick et al. 2000; Al-Deeb & Wilde 2003; Volkmar & Freier 2003; Duan et al. 2004; Ahmad et al. 2005;

Řezáč et al. 2006; Torres & Ruberson 2007; Toschki et al. 2007; Farinós et al. 2008; Kalushkov et al. 2008). Although pitfall trapping is recognized as measuring activity-density rather than absolute density (Thiele 1977), this method is often chosen for its low cost and high capture efficiency (Topping & Sunderland 1992). However, pitfall trapping alone has been noted as a poor indicator of overall abundance, as well as relative abundance of epigeal predators in arable land, often overestimating certain groups (e.g., Lycosidae) and underestimating others (e.g., Linyphiidae) (Lang 2000). Moreover, predator communities captured in pitfall traps are poorly correlated with predation intensity observed in these habitats (Lundgren et al. 2006). Additional characteristics of pitfalls may also affect the efficiency and composition of arthropods captured, including sampling effort (number and duration of pitfall trapping) (Riecken 1999), sampling interval (Schirmel et al. 2010), type of preservative used (Curtis 1980), use of fencing (Holland & Smith 1999), and diameter of pitfall traps (Brennan et al. 2005).

Collection methods for foliar-based spiders included yellow sticky traps, visual searching, whole plant destructive sampling, sweep netting, beat sheet collection, and vacuum-sampling (DVAC suction sampling). Risk-assessment studies in cotton in particular tend to focus on the foliar-based spiders only by using these methods and not epigeal collection methods (e.g., Van Tol & Lentz 1998; Turnipseed & Sullivan 1999; Armstrong et al. 2000; Hagerty et al. 2000, 2005; Moar et al. 2002; Head et al. 2005; Whitehouse et al. 2005); this type of sampling likely skews the data in favor of aerial web-building and foliage-adapted hunting spiders (e.g., Araneidae, Anyphaenidae, Miturgidae) and completely ignores other ground-based web-builders and epigeal hunters (e.g., Linyphiidae, Lycosidae).

Meissle & Lang (2005) determined that the most efficient collecting method for foliar spiders in corn was vacuum-suction sampling, collecting the greatest number and diversity of spiders, plus allowing for lower variation between samples, leading to increased statistical power. In contrast, Amalin et al. (2001) found vacuum-sampling was the least effective sampling method for collecting spiders, particularly for hunting spiders, and spider guilds were not equally collected using this technique. Vacuum-suction sampling has also been found to be an effective collection method of spiders in natural grasslands, although increased vegetation height decreased collection efficiency (Brook et al. 2008); this limitation could have implications for collecting, depending on crop plant architecture.

Our meta-analysis revealed that soil-dwelling and foliar spider communities responded differently to Bt and non-Bt crops in several situations (Fig. 2). Ultimately, using multiple collection methods allows for a more complete examination of the spider community. For example, one study including both foliar and epigeal collections reported a higher mean abundance of spiders based on sweep-net samples, but no significant differences between mean abundances collected by pitfall trapping (Torres & Ruberson 2005). This may indicate that spatial distribution and/or functional niche within an agroecosystem may impact the way that transgenic crops affect subsets of the spider community. Non-target risk-assessment studies of spiders should therefore employ multiple collection methods and get identifications in greater taxonomic detail to obtain an accurate picture of the ecological processes at hand. In some cases, the sampling methods used to collect spiders may affect the ability to detect potential differences in populations between Bt and non-Bt crops. A combination of multiple collection techniques is recommended for the most accurate sampling of spider communities.

6.4 Spider population trends vary spatially and temporally within agroecosystems, and these dynamics are strongly influenced by the crop.—The distribution and expression levels of Bt proteins within a transgenic plant vary depending on the type of Bt toxin, transformation event, gene promoter used, developmental stage, crop phenology, and environmental and geographical effects (Lundgren et al. 2009a). Although the crop plants reviewed here all express Bt toxins, they vary widely in other biological aspects, such as habitat structure and complexity, plant phenology, availability of non-prey resources, microclimatic conditions, and level of disturbance. Therefore, we can predict that the spider communities within each crop type will vary. Uetz et al. (1999) reported differences in the structure of spider guilds within crop fields in the United States. This study presented two distinct dominance structures: those dominated by the guilds defined as "ground runners" (Lycosidae, Dysderidae, and Gnaphosidae) and "web-wanderers" (Linyphiidae and Micryphantidae), which included rice, as well as those crops dominated by "orb weavers" (Araneidae, Tetragnathidae, and Uloboridae) and "stalkers" (Mimetidae, Oxyopidae, and Salticidae), which included corn and cotton. Inherent differences in the spider communities in distinct cropping systems may lead to differential effects of Bt crops on spider assemblages.

7. CONCLUSIONS

Spiders are some of the most diverse and abundant predators in field cropping systems, although their diversity and idiosyncrasies are currently lost in most studies examining Bt crops. Spiders have received little attention in proportion to their abundance and importance as generalist predators in agroecosystems. By combining all spiders together in the analysis of such studies, the ecological value of the data is lost and the potentially differential impact of Bt crops on functionally distinct spider species is subverted. It is therefore essential for risk-assessment literature examining impacts on spiders to identify them to the lowest taxon possible, in order to elucidate how Bt crops are impacting the diverse assemblages of Araneae in transgenic agroecosystems.

Although there are many mechanisms through which Bt crops could affect spiders, there are no consistent negative effects observed in the literature on toxicity of Bt toxins against them. Further study on the uptake of Bt toxins by spiders, pathways to exposure, and the consequences of such are necessary to further our understanding of the interactions between Bt crops and spider assemblages. A remaining question is how Bt-crop-associated changes to agroecosystems affect the ability of spider communities to regulate pest populations.

Several caveats to approaches to sampling spider communities challenge our interpretation of current data involving Bt non-target studies. These include the sampling approach

selected, as well as the region and duration of sampling applied. The diversity of the spider community creates challenges for accurately estimating population densities and can alter perceptions of dominance within spider species assemblages. A multi-tactic strategy will likely give us the best understanding of spider communities within agroecosystems.

Transgenic crop technology has been rapidly adopted in many countries and continues to increase in its planting worldwide. Current transgenic crop development has focused on both the stacking (expression of more than one type of transgene product that target multiple pest species) and pyramiding (expression of more than one type of transgene product that target the same pest) of genes. With the adoption of new crops and expression of additional Bt toxins, risk-assessment is increasingly necessary in understanding how biotechnology may affect ecologically important groups of organisms, such as spiders.

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Reproductive allocation in female wolf and nursery-web spiders

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Abstract. We collected data on maternal mass, clutch mass (reproductive effort), number of offspring, and mean offspring mass from 28 species of Lycosidae (wolf spiders) and five species of Pisauridae (nursery-web spiders) found in Mississippi, USA. Our primary goal was to test for a trade-off between offspring number and offspring size (mass) among wolf and nursery-web spiders, which are sister families. The regression of reproductive effort on maternal mass was highly significant and explained 94% of the variation in reproduetive effort among species and 96% of the variation among genera. The slope of the regression line between maternal mass and total offspring mass was not significantly different from one, suggesting that spiders used a constant proportion of their total energy budget for reproduction regardless of size. Partial correlation and principal components analyses demonstrated a clear trade-off between offspring size and number. Species with large offspring (relative to adult size) produced fewer offspring than expected. Lycosids produced small numbers of large offspring relative to pisaurids, and smaller species of both families are more constrained in the evolution of the offspring size:number continuum than larger ones.

Keywords: Fitness, life history evolution, offspring size, relative reproductive effort, trade-off

Trade-offs between competing energy demands form the basis of life history evolution because individuals have finite amounts of energy that must be divided into conflicting demands for growth, maintenance, and reproduction (Stearns 1992; Roff 2002; Fischer et al. 2006). The reproductive effort of females must then be further divided: females can invest in producing either larger numbers of smaller offspring or fewer offspring of larger size. Larger offspring are typically considered to be more fit, particularly in harsh environments and when inter- or intraspecific competition strongly limits density (Fox et al. 1997; Fox & Czesak 2000; Olsson et al. 2002; Walker et al. 2003). The relationship between offspring size and fitness, however, can be complex, and there are several notable exceptions to the generalization that larger offspring are more fit (e.g., Sinervo et al. 1992; Gomez 2004). Conversely, there is typically strong selection on female fecundity. All else being equal, fitness increases with increases in the number of offspring produced. Optimal clutch size, producing the greatest number of offspring surviving until sexual maturity, is the result of selection on both adults and offspring (Smith & Fretwell 1974; Fox & Czesak 2000). The number of offspring produced should depend on the shape of the function describing the change in fitness for a given change in offspring size.

Many studies have provided both theoretical (e.g., Smith & Fretwell 1974; Lloyd 1987; van Noordwijk & de Jong 1986; Stearns 1992; Roff 2002) and empirical support for the occurrence of such a trade-off among a wide variety of organisms including copepods, fish, birds, bees, plants, and scorpions (e.g. Stearns 1983; Allan 1984; Smith et al. 1989; Elgar 1990; Kim & Thorp 2001; Leishman 2001; Brown 2003) Although the majority of studies have focused on sexually reproducing species, experimental evidence also exists for similar trade-offs in clonally-reproducing plants (Brewer & Platt 1994; Stuefer et al. 2002). To date, the majority of studies have focused on phenotypic trade-offs between offspring number and size. Recent research, however, has shown a genetic basis for the trade-off in some organisms (Snyder 1991; Csezak and Fox 2003; Mappes and Koskela 2004).

The relationship between female mass, offspring mass, and number of offspring among spider species has been previously examined. In an influential paper, Marshall and Gittleman (1994) reviewed data from the literature to examine the relationship between female body mass and clutch/egg size among a taxonomically broad subset of spiders, but did not find support for a trade-off between egg number and mean egg mass. In contrast, our data were collected totally from wild-caught gravid females, from two closely related families with similar reproductive strategies, and from a small geographic area. The current study focused on wolf spiders (Araneae: Lycosidae) and nursery-web spiders (Araneae: Pisauridae), closely related families in the Lycosoidea (Coddington 2005) to: 1) test for the presence of a trade-off between offspring size and number of offspring, 2) describe patterns of reproductive allocation among females, and 3) report life history data for several species for which little or no information exists.

METHODS

Study animals.—Species within both families exhibit two qualities that make them ideal for this study. Females exhibit similar levels of parental care (but see below), and these species are semelparous. Inclusion of iteroparous species can introduce confounding effects of trade-offs between current and future reproduction and current reproduction and future survival (Desouhant et al. 2005; Waelti and Reyer 2007). During 3 yr of field observations on hundreds of spiders, we have never witnessed multiple clutches in nature for these species in Mississippi. Differing levels of parental care have been shown to influence egg investment (Simpson 1995; Ruber et al. 2004).

Species of both families are found in a variety of habitats and are almost exclusively cursorial hunters. Maternal care in both families can be divided into pre- and post-emergence stages. During the pre-emergence stage, wolf spider females

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Table 1.—Summary of some life history data for wolf spiders (Lycosidae) and nursery-web spiders (Pisauridae). The tabled information includes number of individual spiders sampled from each species (*n*), mean mass of females in mg, mean clutch mass in mg, and the mean number of offspring produced per clutch (Fecundity).

Species	*n*	Maternal mass (mg)	Clutch mass (mg)	Fecundity
Lycosidae				
Allocosa funerea (Hentz 1844)	1	17	13	56
Geolycosa fatifera (Hentz 1842)	2	542	177	118
Geolycosa missouriensis (Banks 1895)	1	742	243	133
Gladicosa pulcra (Keyserling 1877)	10	301	185	164
Hogna annexa (Chamberlin & Ivie 1944)	24	246	160	219
Hogna aspersa (Hentz 1844)	4	1288	694	268
Hogna georgicola (Walckenaer 1837)	59	840	517	236
Hogna lenta A	21	599	418	206
Hogna lenta B	11	642	400	569
Hogna wallacei (Chamberlin & Ivie 1944)	5	544	271	228
Hogna watsoni (Gertsch 1934)	1	140	60	60
Pardosa concinna (Thorell 1877)	7	35	22	60
Pardosa milvina (Hentz 1844)	18	20	19	40
Pardosa pauxilla Montgomery 1904	1	12	7	18
Pirata species A	18	12	7	28
Pirata species B	1	35	27	74
Rabidosa carrana (Bryant 1934)	3	592	341	187
Rabidosa hentzi (Banks 1904)	6	250	149	90
Rabidosa punctulata (Hentz 1844)	340	415	194	143
Rabidosa rabida (Walckenaer 1837)	287	599	373	356
Schizocosa avida (Walckenaer 1837)	11	241	105	212
Schizocosa bilineata (Emerton 1885)	2	66	13	28
Schizocosa duplex (Chamberlin 1925)	5	67	43	76
Schizocosa ocreata grp.	11	70	48	80
Schizocosa saltatrix (Hentz 1844)	17	102	75	116
Schizocosa uetzi Stratton 1997	1	73	37	63
Trochosa acompa (Montgomery 1902)	5	88	71	102
Varacosa avara (Keyserling 1877)	11	96	69	73
Pisauridae				
Dolomedes albineus (Hentz 1845)	3	736	650	668
Dolomedes tenebrosus (Hentz 1844)	1	1947	1540	2627
Dolomedes triton (Walckenaer 1837)	2	642	506	1147
Pisaurina dubia (Hentz 1847)	4	50	40	83
Pisaurina mira (Walckenaer 1837)	21	238	268	348

carry egg sacs suspended from their spinnerets, and nursery-web females carry egg sacs in their chelicerae. The post-emergence stage begins after a period of 4–6 wk for wolf spiders and 2–3 wk for nursery-web spiders, when females must tear open the egg sac in order for spiderlings to emerge. In wolf spiders, once the egg sac has been opened the spiderlings emerge and crawl onto their mother's abdomen where they remain for 1–2 wk before dispersing. Nursery-web females, on the other hand, suspend the opened egg sac from a specially constructed 3-dimensional web structure. Emerging spiderlings crawl onto the nursery web and remain there approximately 1–2 wk before dispersing. During this period, the female does not abandon her offspring but remains close, presumably to defend them (but see Kreiter & Wise 2001).

Measuring fecundity.—We opportunistically collected females carrying egg sacs from throughout Mississippi during March–September 2004, 2005, and 2006. Some gravid females were also captured, but individuals not producing an egg sac within 48 h were not used for the study, to avoid the confounding effects of supplemental laboratory feeding. Most of the species included in this study are nocturnal, and we collected at night using a headlamp to locate eye shine. Several of the wolf spider species have not been previously described and we classified them as morphospecies. Altogether, we collected 28 morphospecies of wolf spiders belonging to the following genera: *Allocosa*, *Geolycosa*, *Gladicosa*, *Hogna*, *Pardosa*, *Pirata*, *Rabidosa*, *Schizocosa*, *Trochosa*, and *Varacosa* and five species of nursery-web spiders in the genera *Dolomedes* and *Pisaurina*. We deposited voucher specimens at the Mississippi Entomological Museum, Mississippi State University, Mississippi State. The number of individuals per species collected was highly variable (mean = 27.7, median = 5, Table 1).

We brought females into the laboratory and maintained them individually in plastic containers measuring 22 × 15 cm. The containers were filled with several cm of commercial topsoil, and dried grass stems were added to provide places for spiders to perch. We kept larger individuals of Pisauridae in 38-l aquaria filled with several cm of commercial topsoil and 2–3 large sheets of pine tree bark provided as a substrate for nursery web construction. We misted containers every other day to provide moisture. Females actually carrying egg sacs did not feed, so that the laboratory diet was not a confounding factor on fecundity. Any burrowing behavior, date of egg sac construction, and date of hatching were recorded at each misting or feeding.

We made the following observations for all wolf spiders. When all spiderlings emerged, we weighed the female and her

spiderlings to the nearest milligram. The female was then anesthetized with CO_2 gas and the spiderlings were removed using a soft paint brush. We then weighed the female without the spiderlings and ≥ 30 spiderlings were counted and weighed *en masse*. We collected similar data from nursery-web spiders except that we did not need to anesthetize females or spiderlings. As mentioned earlier, females in this family do not carry emerged offspring but instead create a nursery web eliminating the need for anesthetization to remove offspring. For species producing fewer than 100 spiderlings, all offspring were counted directly. We estimated mean spiderling mass, number of offspring, and total clutch mass using three equations: Total clutch mass = Mass (Female + spiderlings) − Mass (Female); Mean spiderling mass = Total mass of spiderlings counted / Number of spiderlings counted; and Total number of offspring = Total clutch mass/Mean spiderling mass.

Statistical analyses.—We examined the relationships among female body mass and total clutch mass, offspring mass, and number of offspring using least-squares linear regression on natural log-transformed data. The data were transformed in order to prevent one outlier from biasing the regression line and to make the variance in the dependent variable independent of the value of the independent variable (homoscedastic). We also used partial correlation analysis to examine the relationship between offspring mass and number of offspring after removing the effect of maternal body size.

Species data points may not be statistically independent due to traits being shared through common descent; therefore we performed a randomization test using 1,000 permutations to test for a phylogenetic signal. Since the goal of the regression is to look at the variance or invariance of reproductive effort relative to body size, we performed the randomization test on relative reproductive effort. We obtained relative reproductive effort by dividing the total mass of offspring by the mass of the mother (see Reed and Nicholas 2008). We implemented the test using PHYSIGER.M (Blomberg et al. 2003) in MATLAB version 7. We set all branch lengths equal to one, because the topology of the tree for this group is only moderately well known, and estimated branch lengths are unavailable for most species.

To see if the same patterns hold for both taxonomic levels for which we have sufficient replication, all analyses were carried out at both specific and generic levels. As the results were always congruent, regardless of whether species or genera are used, we often provide figures only for the analysis of species.

To determine patterns of reproductive allocation among species and genera we performed principal components analysis (PCA) on the correlation matrix, using varimax rotation. PCA is a multivariate ordination technique appropriate for use in data sets with approximately linear relationships among correlated variables, in this case female mass, offspring mass, and number of offspring. Specifically we were interested in the component that describes explicitly the trade-off between offspring mass and offspring number. PCA analysis was also used to test for differences in reproductive allocation between nursery-web and wolf spiders.

RESULTS

The vast majority of variation in total reproductive energy expenditures can be explained simply by the mass of the mother. Female mass and total clutch mass were positively and highly significantly correlated, with 94% of the variation in total clutch mass explained by female mass at the specific level (i.e., mean value for each species) ($F = 519.6$, $df = 32$, $P < 0.001$, Fig. 1a) and 96% at the level of genera (i.e., mean value for each genus) ($F = 222.4$, $df = 11$, $P < 0.001$, Fig. 1b).

A randomization test performed on relative reproductive effort failed to show a significant phylogenetic signal ($P = 0.11$). Because of the lack of phylogenetic signal, the narrow taxonomic focus of the study, and a poorly resolved phylogeny of these species, we opted not to perform a phylogenetically-correlated regression analysis.

The slope of the regression line between the natural log of female mass and the natural log of total offspring mass is of particular interest for life history evolution and the evolution of body size, as it relates to the efficiency of energy conversion in similar organisms of varying mass. In the current study, the regression line was not significantly different from one ($b = 0.98 \pm 0.04$ at the specific level and $b = 0.92 \pm 0.06$ at the generic level). This indicates that these species and genera use a constant proportion of their energy for reproduction regardless of body size.

Female mass was also positively correlated with number of offspring and mean offspring mass. Female mass explained 70% of the variation in number of offspring at the specific level ($F = 73.4$, $df = 32$, $P < 0.0001$, Fig. 2) and 69% of the variation in number of offspring at the generic level ($F = 22.1$, $df = 12$, $P = 0.0008$). Female mass explained 59% of the variation in mean offspring mass at the specific level ($F = 44.6$, $P < 0.0001$, Fig. 3) and 71% of the variation in mean offspring mass at the generic level ($F = 24.7$, $df = 12$, $P < 0.001$). Like Marshall and Gittleman (1994), we found negative allometry between maternal size and offspring size, so that smaller spiders tend to produce relatively larger offspring. Partial correlation analysis between number of offspring and offspring mean mass showed that number and size of offspring were significantly and negatively correlated at the specific level ($r = -0.82$) and at the generic level ($r = -0.88$).

We obtained similar results through principal components analyses. Axis 1 explained 77.6% of the variation among species and is positively correlated with female mass ($r = 0.992$), offspring mass ($r = 0.799$), and offspring number ($r = 0.841$) (Fig. 4). Similarly, the first principal component explained 80.7% of the variation among genera. Axis 1 was highly positively correlated with female mass ($r = 0.996$), mean offspring mass ($r = 0.849$), and offspring number ($r = 0.842$). [In other words, when female mass is included as a variable, the resulting pattern is one of species or genera with larger females having larger offspring and larger numbers of offspring.]

Axis 2 explained 21.5% of the variation among species and is positively correlated with offspring mass ($r = 0.597$) and negatively correlated with offspring number ($r = -0.536$). Axis 2 was only very weakly related to female mass ($r = -0.027$).

The second component explained 18.8% of the variation among genera. Axis 2 is positively correlated with mean offspring mass ($r = 0.537$) and negatively correlated with

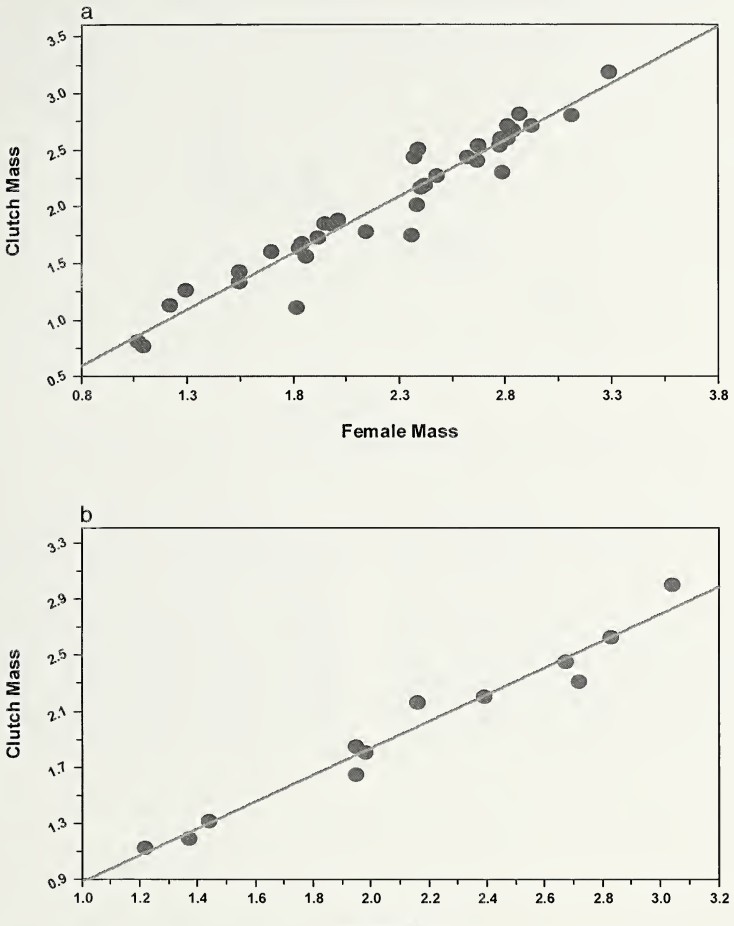

Figure 1.—Least squares linear regression using $\log_{10}$ female mass (in mg) as the independent variable and $\log_{10}$ total offspring mass (in mg) as the dependent variable for *a*) specific means and *b*) generic means. The regressions are highly significant ($P < 0.001$ for both) and explain 94% and 96% of the variation in total clutch mass, respectively.

number of offspring ($r = -0.525$), but shows a very weak relationship to female mass ($r = -0.006$).

Axis 2 was positively correlated with offspring mass and negatively correlated with number of offspring in both of the above analyses. In other words, Axis 2 is reduced into a new variable or component that explicitly describes the inherent trade-off between offspring mass and number of offspring. However, the variation in which we are most interested is reflected in the PCA residuals for offspring mass and PCA residuals for offspring number when regressed against female body size. Both show a high correlation with Axis 2. At the specific level, Axis 2 is positively correlated with residual offspring mass ($r = 0.92$; $P < 0.001$) and negatively correlated with residual offspring number ($r = -0.96$; $P < 0.001$) (Fig. 5). At the generic level, Axis 2 is positively correlated with the residuals from the linear regression of offspring mass onto female mass ($r = 0.93$; $P < 0.001$) and negatively correlated with residuals from the linear regression of offspring number onto female mass ($r = -0.91$; $P < 0.001$). Thus, we feel confident that Axis 2 represents real patterns of reproductive allocation among these species and genera.

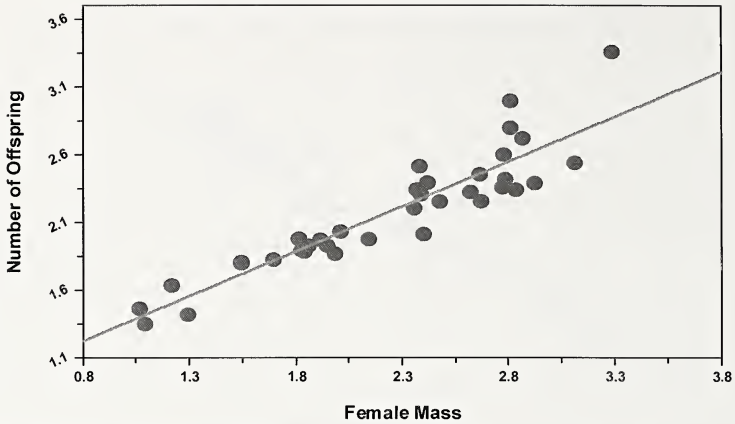

Figure 2.—The relationship between $\log_{10}$ female mass and $\log_{10}$ number of offspring. Female mass explained 70% of the variation in number of offspring at the specific level using least-squares linear regression ($P < 0.0001$).

We also separately regressed Axis 1 against Axis 2 for lycosid and pisaurid spiders (see Fig. 4). The slope for the wolf spiders was negative (-0.52 ± 0.13) and the slope for the nursery-web spiders was positive (0.59 ± 0.34). The two slopes were significantly different from each other (ANCOVA; $F_{2,31} = 4.76$, $P < 0.025$). Thus, lycosids produce smaller numbers of larger offspring relative to pisaurids. Although not statistically testable because of insufficient sample size, there is an obvious bifurcation of the distribution at larger female body sizes for the wolf spiders and nursery web spiders in their allocation patterns. At smaller sizes the two families appear more similar in their allocations patterns.

DISCUSSION

Here we report three major results from our study. 1) Female wolf spiders and female nursery-web spiders have diverged in their reproductive allocation, with wolf spiders generally producing relatively small numbers of large offspring compared to nursery-web spiders. 2) In both families, reproductive effort (total clutch mass) increases in a log-linear fashion with female mass. Larger wolf and nursery-web spiders use neither a larger or smaller portion of their total energy budget for reproduction. 3) In both families, offspring size is negatively correlated with offspring number among species and among genera, indicating a trade-off between

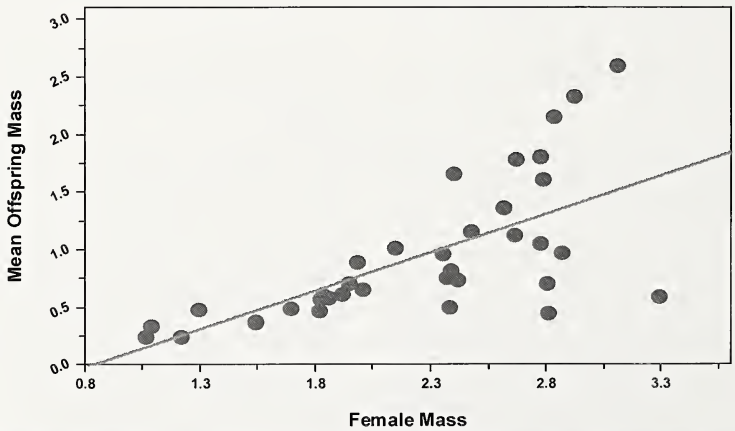

Figure 3.—The relationship between $\log_{10}$ female mass and $\log_{10}$ mean offspring mass. Female mass explained 59% of the variation in mean offspring mass at the specific level ($P < 0.0001$).

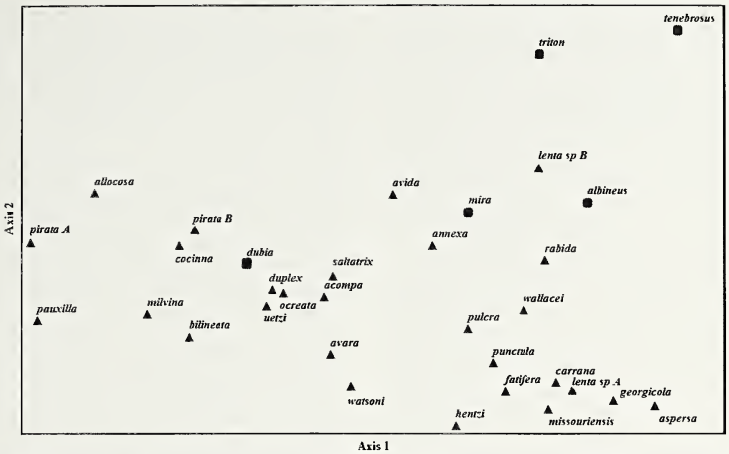

Figure 4.—Principal components analysis demonstrating the trade-off between offspring size and offspring number. Triangles represent species of wolf spider and squares represent species of nursery-web spiders. Axis 1 represents female mass ($r = 0.99$) and Axis 2 is positively associated with number of offspring ($r = 0.60$) and negatively correlated with mean offspring mass ($r = -0.54$). Further, Axis 2 is positively correlated residual offspring mass ($r = 0.92$; $P < 0.001$) and negatively correlated with residual offspring number ($r = -0.96$; $P < 0.001$). Thus, Axis 2 accurately represents patterns of reproductive allocation and demonstrates a trade-off between the two. Noteworthy is the bifurcation of the distribution at larger sizes and the divergence of wolf spiders and nursery web spiders in their allocation patterns.

offspring size and offspring number. Below we provide a brief theoretical background and then elaborate on our findings.

Life history theory predicts a potential trade-off between offspring number and offspring size because there is a finite amount of energy available for reproduction. Thus, all else being equal, selection for larger offspring is predicted to result in a smaller number of offspring (reviewed by Fox and Czesak 2000). Variation in total energy available reflects differences in energy acquisition among different species and among individuals within a species. Differences at the interspecific level may be due to both phylogenetic and environmental influences (Brown 2003). In a seminal paper, van Noordwijk

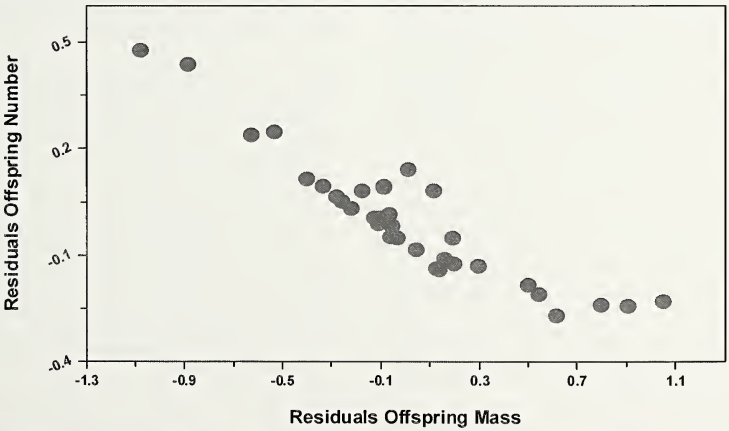

Figure 5.—Residuals from the regressions in Figure 2 and Figure 3 plotted against each other. The regression showed a significant negative relationship between residual offspring mass and residual number of offspring ($r = -0.92$; $P < 0.0001$). The strong negative relations demonstrates a size:number trade-off. Species with larger than average offspring (relative to adult size) also produce fewer offspring that average (relative to adult size).

and de Jong (1986) produced a simple model predicting that when there is variation among individuals in the amount of resources available, the trade-off between individual expenditures will be obscured. Although the van Noordwijk and de Jong model seeks to explain trade-offs at the intraspecific level, the same logic necessarily applies at the interspecific level. Some species will have more energy available for reproduction, on average, than others (i.e., larger species will have more energy). Thus, demonstration of a trade-off in this paper is facilitated by the fact that the species studied use a similar proportion of their available resources for reproduction when averaged across individuals of that species.

Our null hypothesis was that all of the variance in reproductive effort, clutch size, and mean mass of spiderlings could be explained simply by maternal mass. Our data demonstrate that indeed almost all of the variation among the species and genera in total reproductive effort can be explained by mean female mass alone. However, the relationships between female body mass and offspring size or between female body mass and offspring number are considerably more variable. Thus, there exists variation in patterns of reproductive allocation among these species. Our interpretation of the principal components analyses is that most of the variation occurs among the larger species. In particular, the reproductive allocation patterns are quite different between the larger species of pisaurids and lycosids. Pisaurids with large mean female mass tend to produce many small offspring, while similarly-sized lycosids produce fewer, larger offspring. One exception to this pattern is *Hogna lenta B* which has an allocation pattern similar to the pisaurids.

The lack of variation in offspring size among species with small mean female mass suggests some constraint. Female spiders have partially sclerotized reproductive parts, which could constrain resource allocation to a minimum egg size in smaller species regardless of whether the optimal size is a larger clutch of smaller eggs (Foelix 1982). Marshall and Gittleman (1994) found a similar pattern and suggest limits to surface-to-volume ratio of eggs or a minimum size for offspring based on available prey or avoiding desiccation.

Our null hypothesis was that the scaling of maternal mass to clutch mass would be isometric, so that for each incremental increase in size a species would increase its reproductive effort. Indeed, the slope of the regression line between female mass and clutch mass was not significantly different from one. A slope of one suggests a constant relative reproductive effort ($63 \pm 3\%$ of female mass), where larger spiders do not invest a larger or smaller proportion of their available energy than smaller spiders. Our result is consistent with the results of Marshall and Gittleman (1994), who also found a slope of one for a taxonomically broader sampling from the literature. This result is also consistent with results for individuals within species for *Nephila clavipes* (Linnaeus 1767) and *N. pilipes* (Fabricius 1793) (Higgins 1992, 2000, 2002) and also for *Rabidosa punctulata* (Hentz 1844) and *R. rabida* (Walckenaer 1837) (Reed and Nicholas 2008).

Most importantly, we tested for a trade-off between clutch size and offspring size (as per Marshall and Gittleman 1994). We found, among the species of wolf and nursery-web spiders we studied, a strong trade-off between offspring size and number of offspring. This result differs from that of Marshall and Gittleman (1994) who found no such trade-off. Possible reasons for the different conclusion are numerous. Marshall and Gittleman assayed a far broader taxonomic sample than we did, had smaller sample sizes within each species, and included species that are not semelparous. Further, Marshall and Gittleman secured the vast majority of their data from the literature and many of them may have been based on laboratory-reared individuals. For example, *Rabidosa punctulata* is listed by Marshall and Gittleman as producing a mean of 2.5 clutches of eggs. However, four years of mark and recapture data in our Mississippi populations (Reed et al. 2007a,b; Reed and Nicholas 2008) failed to reveal a second egg sac in a wild-caught females of this species. Therefore, the distinct results could be due to greater statistical power present in our less-noisy data set, trade-off being obscured by differences in reproductive behavior (e.g., amount of maternal care), because differences in the broader range of behaviors in the more diverse taxonomic group can confound measures of the amount of energy actually spent on reproduction.

We recommend that future studies on reproductive allocation in spiders focus on large samples of individuals with a narrow taxonomic focus. Most (74%) of the variation in relative reproductive effort was among individuals not among taxonomic groupings in our study. If the trade-off is tested in several well-studied but phenotypically diverse groups of spiders, patterns may become evident concerning what factors influence the presence of the trade-off or might obscure existing trade-offs among behaviorally heterogeneous groups. We also suggest that more data are needed to support or refute the conclusion that the relationship between maternal mass and clutch mass at the species level is the same at diverse taxonomic levels, as suggested here. If such an isometric scaling is shown consistently, theoretical studies might be useful to examine the physiological or evolutionary basis for the constraint.

ACKNOWLEDGMENTS

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Two new *Draconarius* species and the first description of the male *Draconarius molluscus* from Tiantangzhai National Forest Park, China (Araneae: Agelenidae: Coelotinae)

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Abstract. Three *Draconarius* species collected from the Tiantangzhai National Forest Park, China are studied, including two new species, *D. peregrinus* sp. nov. and *D. tiantangensis* sp. nov. The male *D. molluscus* (Wang et al. 1990) is reported for the first time.

Keywords: Taxonomy, diagnosis, new species

Draconarius Ovtchinnikov 1999 is one of the most diverse genera in the subfamily Coelotinae (Liu & Li 2009). At present, a total of 204 *Draconarius* species are known worldwide, among which 110 are recorded from China (Platnick 2010; Wang 2010). Many *Draconarius* species are currently described from only male or female specimens. Most of those described from both sexes are only based on a small number of individuals, and some sexes may be incorrectly matched. As a result, a morphological phylogenetic analysis at this moment would be challenging, Wang divided these species into seven groups in 2003, but the other unplaced species remain to be sorted (Wang 2003).

Recent field surveys in Tiantangzhai National Forest Park, China have yielded three *Draconarius* species, which we describe in the current paper. Tiantangzhai National Forest Park (Fig. 40), located in the Dabie Mountains between Hubei and Anhui provinces in China, is part of the watershed of Yangtze River and Huai River. With an average elevation of 1000 m, its highest peak reaches 1729 m, the second highest peak in Dabie Mountains. Tiantangzhai has a subtropical climate, with typical mild climate accompanied by abundant rainfall. The fauna is typical for the transition zone between the Palaearctic and Oriental regions: e.g., Palearctic Otididae, such as bustard; Oriental Suidae and Viverridae, such as boar and small Indian civet. Nearly 1400 species of plants and 600 species of animals exist here, including giant salamanders and leopards. Tiantangzhai is the last piece of virgin habitat in eastern China.

METHODS

All specimens used in the current study are deposited in the College of Life Sciences, Hubei University. We examined specimens with an Olympus SZX16 stereomicroscope. Further details were studied with an Olympus BX51 compound microscope. We examined and illustrated male palps and female epigyna after dissecting them from the spider bodies. All illustrations were made using rotring isograph pens (0.20, 0.30 mm) on parchment papers.

All measurements, obtained using an Olympus SZX16 stereomicroscope, are given in millimeters. Eye diameters were taken at the widest point. The total body length does not include the length of the chelicerae or spinnerets. The leg measurements are shown as total length (femur, patella + tibia, metatarsus, tarsus). The terminology used in the text and in the figure legends mainly follows Wang (2002) and Liu & Li (2010). Photos of male palp and female epigynum will be submitted to and available from Li & Wang (2009).

Abbreviations used in the text and figures are: A = atrium; ALE = anterior lateral eye; AME = anterior median eye; AME–ALE = distance between AME and ALE; AME–AME = distance between AMEs; ALE–PLE = distance between ALE and PLE; C = conductor; CD = copulatory duct; CDA = dorsal apophysis of the conductor; CF = cymbial furrow; E = embolus; ET = epigynal teeth; FD = fertilization duct; H = hood; LTA = lateral tibial apophysis; MA = median apophysis; PA = patellar apophysis; PLE = posterior lateral eye; PME = posterior median eye; PME–PLE = distance between PME and PLE; PME–PME = distance between PMEs; RTA = retrolateral tibial apophysis; S = spermathecae; SH = spermathecal head; ST = subtegulum; T = tegulum; TS = tegular sclerite.

TAXONOMY

Agelenidae C.L. Koch 1837
Draconarius Ovtchinnikov 1999
Draconarius molluscus Wang et al. 1990
Figs. 1–12

Coelotes molluscus Wang et al. 1990:214, figs. 86, 87 (♀); Song et al. 1999:376, figs. 221G, H (♀).
Draconarius molluscus Wang 2002:67 (♀); Wang 2003:539, figs. 42A–B, 96D (♀).

Materials examined.—Tiantangzhai National Forest Park, China, Xin Xu and Haijuan Xie, 3♂, 6♀ (27 September 2009), 1♀ (28 September 2009), 1♂, 3♀ (29 September 2009).

Diagnosis.—This species is similar to *D. lutulentus* (Wang et al. 1990), but can be distinguished by the long cymbial furrow (more than half of cymbial length), the short, square tegular sclerite, the absence of epigynal teeth and the long, anteriorly converging spermathecal stalks (Figs. 1–12).

Description.—*Male:* Total length 5.57–5.80. Prosoma 2.82 long, 1.98 wide; opisthosoma 2.62 long, 1.89 wide. Eye: AME 0.15; ALE 0.17; PME 0.17; PLE 0.18; AME–AME 0.05; AME–ALE 0.03; ALE–PLE 0; PME–PME 0.08; PME–PLE 0.07. Clypeus height 0.23. Leg formula: IV, I, II, III; leg: I: 10.68 (2.68, 3.70, 2.73, 1.57); II: 9.29 (2.45, 3.00, 2.48, 1.36); III: 8.56 (2.42, 2.78, 2.04, 1.32); IV: 10.78 (2.85, 3.61, 3.03, 1.29). Chelicerae with 3 promarginal and 3 retromarginal teeth. Patellar apophysis long, sword-shaped; RTA short, less than half tibial length, with distal end protruding beyond

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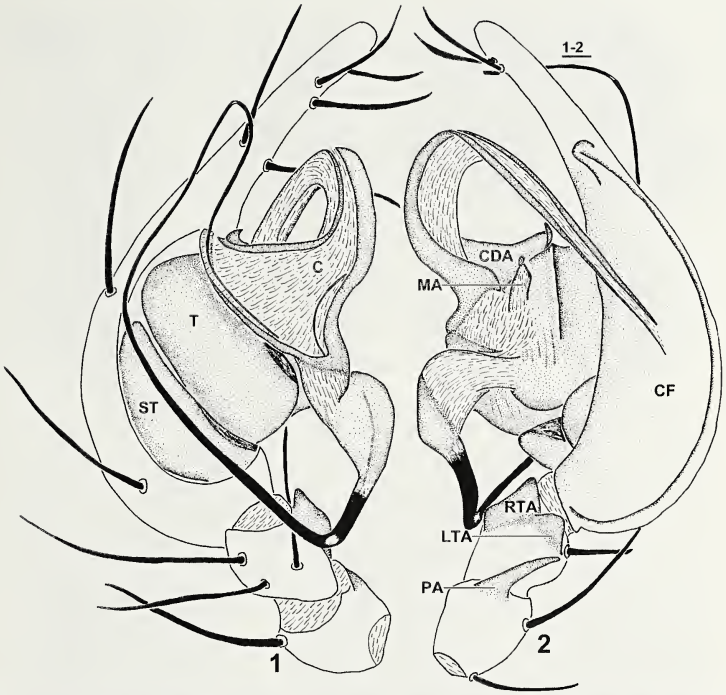


Figures 1, 2.—*Draconarius molluscus*, male. 1. Palp, prolateral view; 2. Palp, retrolateral view. Scale line = 0.1 mm.

distal tibia; lateral tibial apophysis large, closed to RTA; cymbial furrow more than half of cymbial length; conductor long, slender, slightly curved, with large basal lamella; conductor dorsal apophysis long, with sharp distal end; median apophysis short, slightly curved distally; embolus filiform, long, originating retrolaterally (Figs. 1–3, 6, 8–10).

Female: See description of Wang (2003).

Relationships.—*Draconarius molluscus* is a member of the lutulentus-species group.

Distribution.—China (Anhui, Hubei, Jiangxi).

Draconarius peregrinus sp. nov.
Figs. 13–27

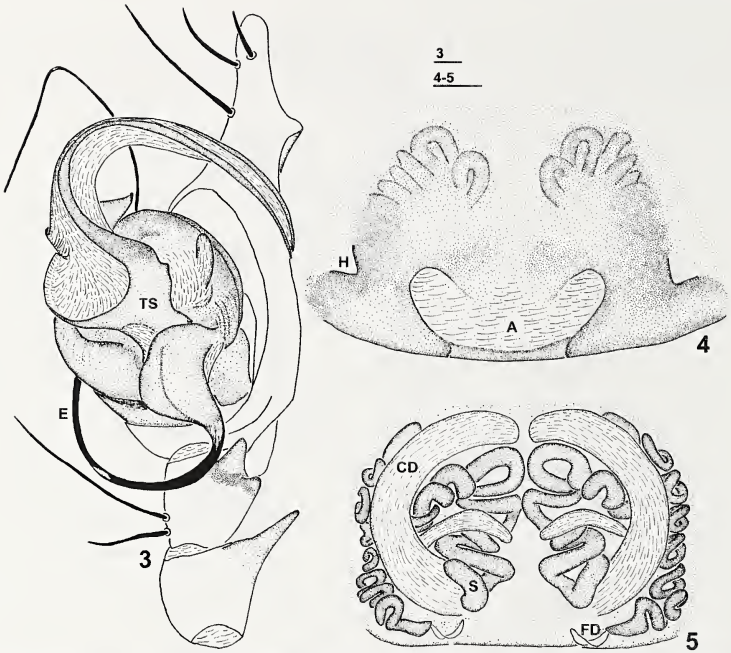
Type species.—Holotype ♂, Tiantangzhai National Forest Park, China, 26 September 2009, Xin Xu and Haijuan Xie. Paratypes: 1♂, 3♀ (same date as holotype); 3♀ (27 September 2009).

Etymology.—The specific name is taken from the Latin adjective *peregrinus*, meaning "bizarre", referring to the strange and unique distal part of embolus.

Diagnosis.—The male of this new species is similar to *Draconarius magicus* (Liu et al. 2010) in having a broad and biforked embolus, but can be distinguished from it by the absence of a patellar apophysis, and the large lateral tibial apophysis situated close to RTA; the proximally originating embolus in the male. The female can be distinguished from other *Draconarius* by the large, folded, wrinkled copulatory ducts and the long spermathecal stalks, which are coverd by copulatory ducts visible in dorsal view (Figs. 13–27).

Description.—*Male:* Total length 6.84–8.51. Prosoma 3.79 long, 2.30 wide; opisthosoma 4.19 long, 2.80 wide. Eye: AME 0.13; ALE 0.22; PME 0.19; PLE 0.20; AME–AME 0; AME–ALE 0.03; ALE–PLE 0; PME–PME 0.05; PME–PLE 0.10. Clypeus height 0.16. Leg formula: IV, I, II, III; leg: I: 7.99 (2.50, 2.68, 1.67, 1.14); II: 7.54 (2.14, 2.31, 1.87, 1.22); III: 6.41 (1.67, 2.34, 1.33, 1.07); IV: 9.92 (2.71, 3.28, 2.66, 1.27). Chelicerae with 3 promarginal and 2 retromarginal teeth. Patellar apophysis absent; RTA short, approximately half tibial length; lateral tibial apophysis broad, closed to RTA; cymbial furrow long, more than half of cymbial length; conductor short, simple; dorsal apophysis moderately large; median apophysis long and slender, spoon-like; embolus broad, originating retrolaterally, with slender bifurcate distal part, one apex sword-shaped, the other oval-shaped (Figs. 13–16, 20, 22–24).

Female: Total length 6.79–10.07. Prosoma 3.90 long, 2.34 wide; opisthosoma 4.14 long, 2.86 wide. Eye: AME 0.14; ALE 0.23; PME 0.20; PLE 0.21; AME–AME 0; AME–ALE 0.04; ALE–PLE 0; PME–PME 0.07; PME–PLE 0.12. Clypeus height 0.19. Leg formula: IV, I, II, III; leg: I: 8.19 (2.45,



Figures 3–5.—*Draconarius molluscus*. 3. Male palp, ventral view; 4. Female epigynum, ventral view; 5. Female epigynum, dorsal view. Scale line = 0.1 mm.

2.85, 1.66, 1.23); II: 6.98 (2.07, 2.44, 1.44, 1.03); III: 6.35 (1.63, 2.26, 1.37, 1.09); IV: 10.00 (2.71, 3.37, 2.76, 1.16). Chelicerae with 3 promarginal and 2 retromarginal.

Epigynal teeth small, situated anteriorly laterad of the atrium; atrium broad; copulatory ducts broad, folded, originating anteriorly or posteriorly, close together; spermathecal stalks long, totally hidden by the copulatory ducts; spermathecal heads small, also totally hidden by the copulatory ducts; spermathecae oval, widely separated (Figs. 17–19, 21, 25–27).

Relationships.—*Draconarius peregrinus* sp. nov. is considered congeneric with the type species of the genus *Draconarius*, as it exhibits two retrolateral teeth; a long cymbial furrow; spoon-like median apophysis; dorsal apophysis of conductor present. Female epigynum with two epigynal teeth, copulatory ducts broad. However, the bifurcate distal part of the embolus makes its generic placement questionable.

Distribution.—China (Hubei, Anhui).

Draconarius tiantangensis sp. nov.
Figs. 28–39

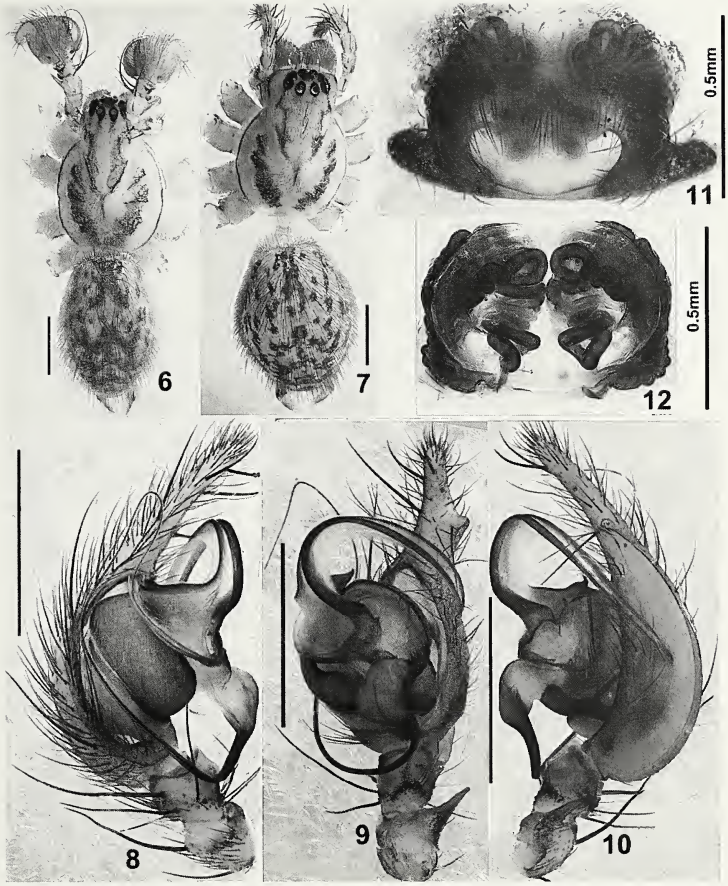
Type species.—Holotype ♂, Tiantangzhai National Forest Park, China, 27 September 2009, Xin Xu and Haijuan Xie. Paratypes: 2♀ (same date as for holotype).

Etymology.—The specific name is an adjective, referring to the type locality, Tiantangzhai.

Diagnosis.—This new species is similar to *Draconarius. aspinatus* (Wang et al. 1990) in the absence of a patellar apophysis, the presence of long cymbial furrow, the simple conductor, the small atrium and the simple large spermathecae, but can be distinguished by the significantly smaller body, with a male length of 4.32mm, the latter is 10 mm long (Wang et al. 1990); and the lateral apophysis broad, close to RTA in this new species, but small, far from RTA in *D. aspinatus* (Figs. 28–39).

Description.—*Male:* Total length 4.32. Prosoma 2.01 long, 1.44 wide; opisthosoma 2.10 long, 1.34 wide. Eye: AME 0.08; ALE 0.13; PME 0.14; PLE 0.15; AME–AME 0; AME–ALE 0; ALE–PLE 0; PME–PME 0.03; PME–PLE 0.02. Clypeus height 0.14. Leg formula: IV, I, II, III; leg: I: 5.17 (1.39, 1.69, 1.25, 0.84); II: 4.86 (1.39, 1.57, 1.13, 0.77); III: 4.51 (1.19, 1.48, 1.19, 0.65); IV: 6.37 (1.66, 2.09, 1.84, 0.78). Chelicerae with 3 promarginal and 2 retromarginal teeth. Patellar apophysis absent; RTA long, with distal end extending beyond tibia; lateral tibial apophysis broad, close to RTA; cymbial furrow long, more than half of cymbial length; conductor simple; dorsal apophysis of conductor semicircular in the ventral view; median apophysis slender, elongated, spoon-shaped; embolus long, filiform, originating proximally (Figs. 28–30, 33, 35–37).

Female: Total length 4.36–4.97. Prosoma 1.85 long, 1.47 wide; opisthosoma 2.16 long, 1.41 wide. Eye: AME 0.09; ALE



Figures 6–12.—*Draconarius molluscus*. 6. Male, dorsal view; 7. Female, dorsal view; 8. Male palp, prolateral view; 9. Male palp, ventral view; 10. Male palp, retrolateral view; 11. Female epigynum, ventral view; 12. Female epigynum, dorsal view. Scale line = 1.0 mm unless stated otherwise.

0.14; PME 0.15; PLE 0.16; AME–AME 0; AME–ALE 0; ALE–PLE 0; PME–PME 0.05; PME–PLE 0.04. Clypeus height 0.15. Leg formula: IV, I, II, III; leg: I: 4.94 (1.42, 1.74, 1.05, 0.73); II: 4.23 (1.26, 1.51, 0.80, 0.66); III: 4.14 (1.23, 1.28, 0.99, 0.64); IV: 5.17 (1.41, 1.86, 1.24, 0.66). Chelicerae with 3 promarginal and 2 retromarginal. Epigynal teeth short, widely separated, situated anteriorly laterad of atrium; atrium small, situated anteriorly near epigastric furrow; copulatory ducts small, originating posteriorly; spermathecal heads long and slender; spermathecae large, close to each other (Figs. 31, 32, 34, 38, 39).

Relationships.—*Draconarius tiantangensis* sp. nov. exhibits a typical *Draconarius* in having a lateral tibial apophysis; a long cymbial furrow; a conductor dorsal apophysis; a spoon-shaped median apophysis; and a long embolus. The female epigynum with epigynal teeth short, widely separated; spermathecae broad. *Draconarius tiantangensis* sp. nov. is a member of the *venustus*-species group.

Distribution.—China (Hubei).

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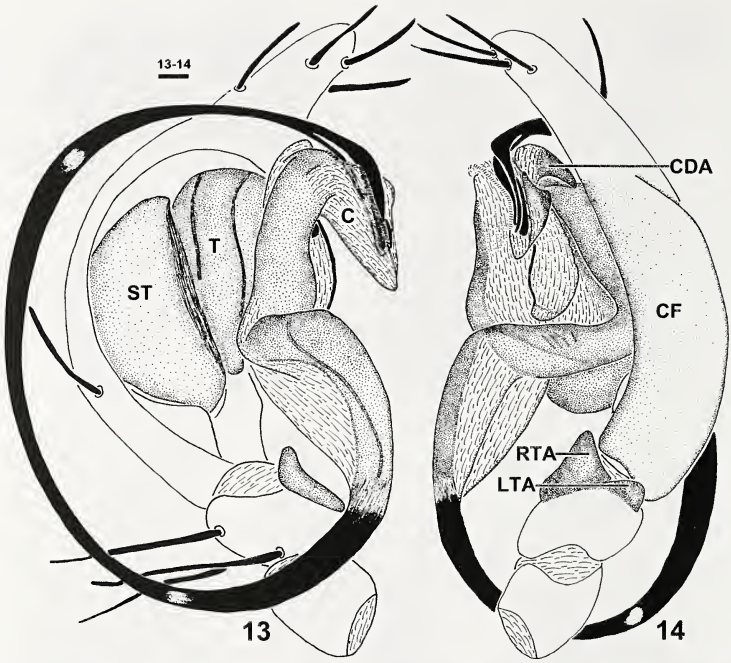
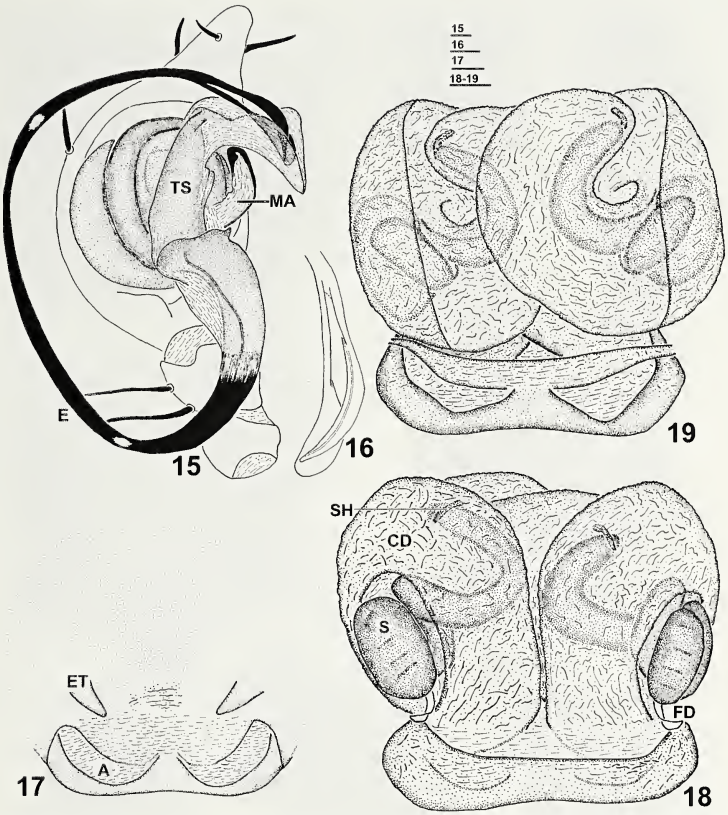
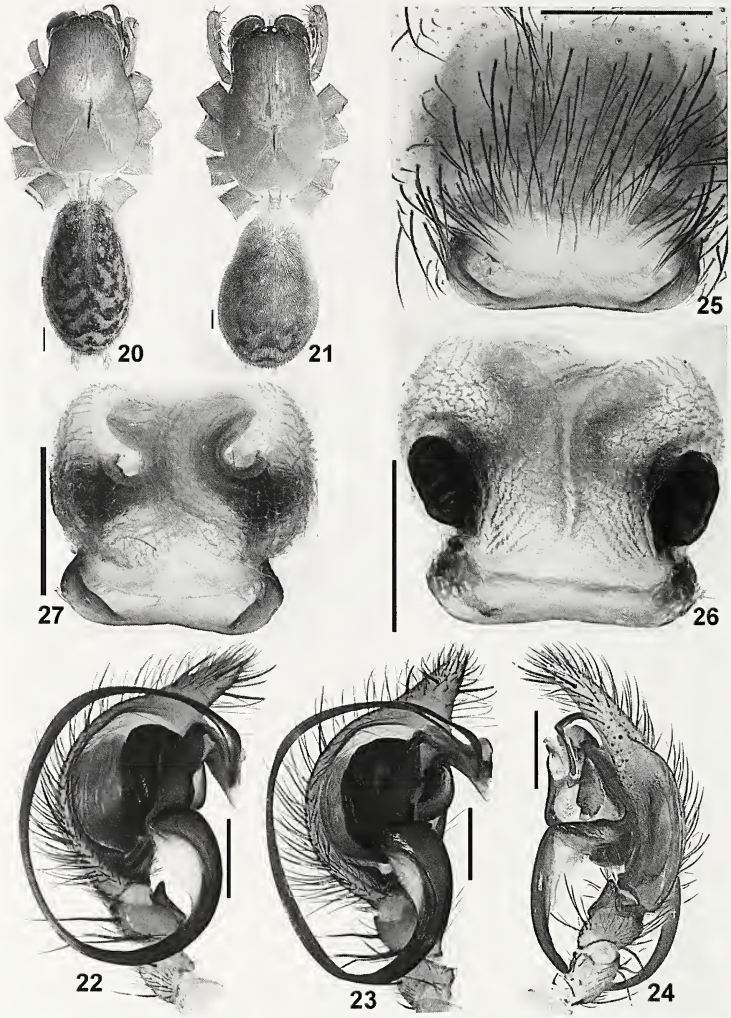


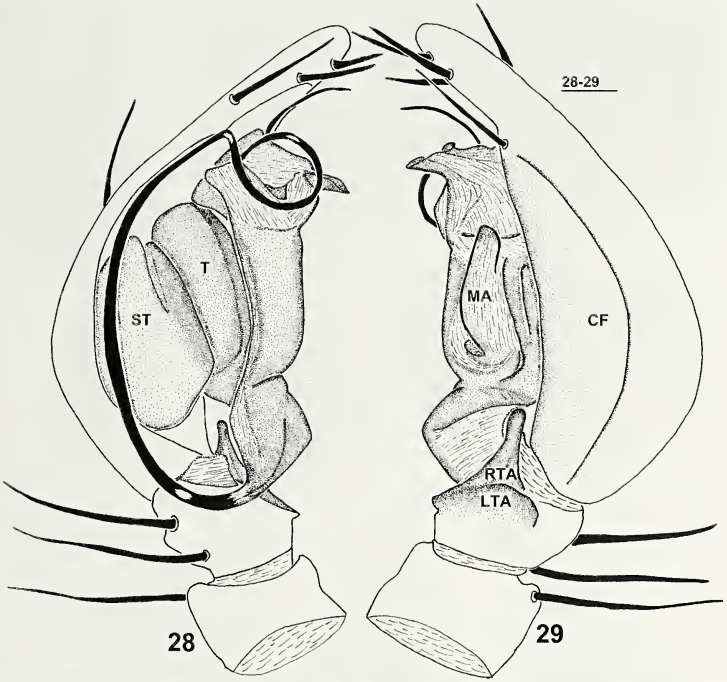
Figures 13, 14.—*Draconarius peregrinus* sp. nov., male. 13. Palp, prolateral view; 14. Palp, retrolateral view. Scale line = 0.1 mm.



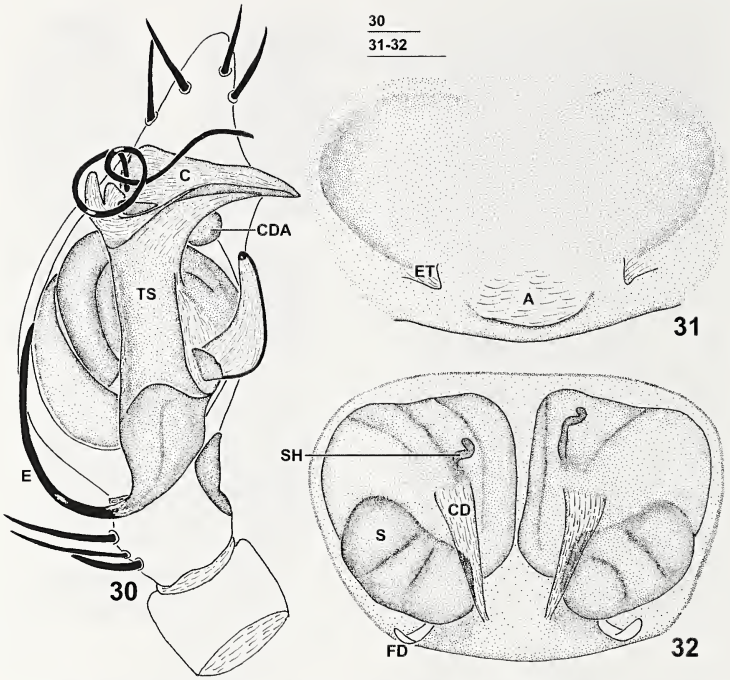
Figures 15–19.—*Draconarius peregrinus* sp. nov. 15. Male palp, ventral view; 16. distal part of embolus, ventral view; 17. Female epigynum, ventral view; 18. Female epigynum, dorsal view; 19. Vulva, ventral view. Scale line=0.1mm.



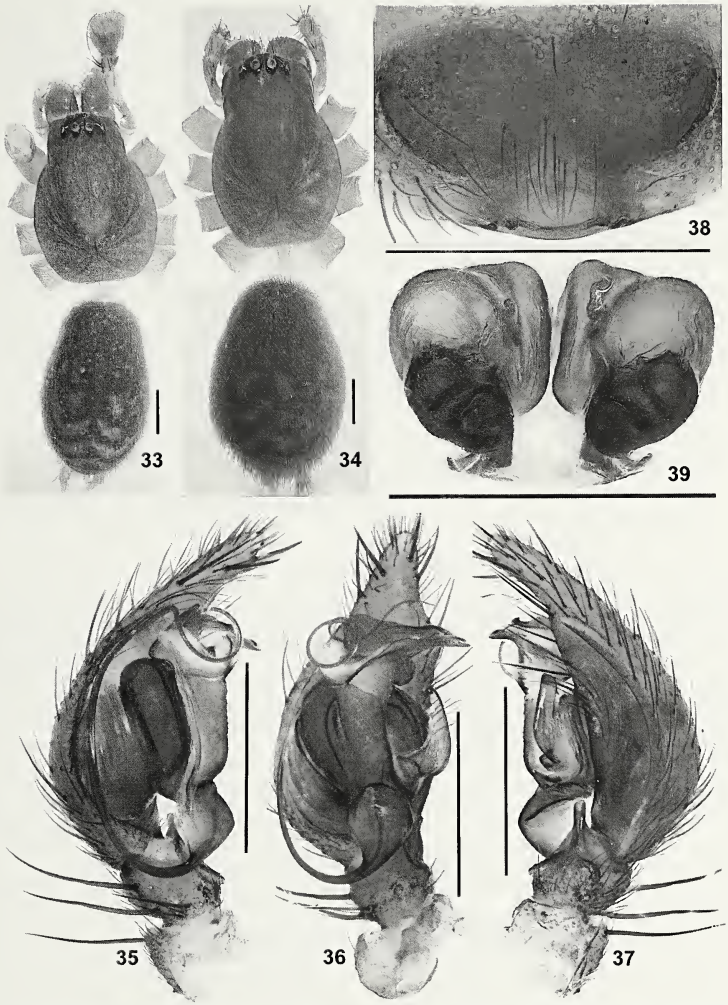
Figures 20–27.—*Draconarius peregrinus* sp. nov. 20. Male, dorsal view; 21. Female, dorsal view; 22. Male palp, prolateral view; 23. Male palp, ventral view; 24. Male palp, retrolateral view; 25. Female epigynum, ventral view; 26. Female epigynum, dorsal view; 27. Vulva, ventral view. Scale line=0.5mm.



Figures 28, 29.—*Draconarius tiantangensis* sp. nov., male. 28. Palp, prolateral view; 29. Palp, retrolateral view. Scale line =0.1mm.



Figures 30–32.—*Draconarius tiantangensis* sp. nov. 30. Male palp, ventral view; 31. Female epigynum, ventral view; 32. Female epigynum, dorsal view. Scale line = 0.1 mm.



Figures 33–39.—*Draconarius tiantangensis* sp. nov. 33 Male, dorsal view; 34. Female, dorsal view; 35. Male palp, prolateral view; 36. Male palp, ventral view; 37. Male palp, retrolateral view; 38 Female epigynum, ventral view; 39. Female epigynum, dorsal view. Scale line = 0.5 mm.

Figure 40.—Location map of the Tiantangzhai National Forest Park.

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Impacts of temperature, hunger and reproductive condition on metabolic rates of flower-dwelling crab spiders (Araneae: Thomisidae)

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Abstract. Temperature strongly affects spider metabolic rate. Consequently, quantifying a species' temperature-metabolism relationship is useful in evaluating consequences of choices that affect body temperature. Body size also influences metabolic rate, and body size in spiders is strongly impacted by feeding and reproductive condition. Using adult female crab spiders, *Misumenoides formosipes* Walckenaer 1837 and *Mecaphesa asperata* (Hentz 1847) (formerly *Misumenops asperatus*) acclimated to field ambient conditions, I measured standard metabolic rates (SMR) over an ecologically relevant temperature range (10–40° C). I controlled hunger and reproductive condition of *M. formosipes* using starved (25 days post-feeding) or fed (7 days post-feeding) spiders, and virgin or mated spiders; in experiments with *M. asperata*, I used fed spiders of unknown reproductive status. Temperature strongly affected crab spider SMR, and both species showed similar temperature-SMR relationships. *Mecaphesa asperata* displayed equivalent temperature coefficients (Q_{10}s – the factor by which a physiologic process changes with temperature) for SMR across the experimental temperature range, while *M. formosipes* had significantly higher Q_{10} at low temperature than at mid-range or high temperature; Q_{10}s of the two species reflected previously determined impacts of temperature on hunting performance. Influence of hunger-reproductive condition on SMR of *M. formosipes* depended on how I accounted for body size; regardless of method, gravid spiders did not show elevated metabolic rate. Lastly, I combined crab spider SMR data with published SMR data to generate mass-metabolism equations for spiders; mass-scaling exponents approximated 0.67.

Keywords: Body size, mass scaling, Q_{10}, SMR, starvation

Respiratory metabolism describes an animal's cost of living. In spiders that ambush prey using a sit-and-wait strategy rather than a web trap, foraging costs approximate standard metabolic rates (Riechert & Harp 1987). Consequently, such spiders may serve as useful models for elucidating the impacts of various factors on metabolic rate and subsequent fitness.

Temperature and body size are the most important variables affecting metabolic rate (Meehan 2006; Gillooly et al. 2001). Temperature is a keystone variable that exerts pervasive effects at all levels of biological organization (Hochachka & Somero 1984), and its impact on an animal's physiological capacities ultimately affects performance and fitness (Huey & Kingsolver 1989). The influence of temperature on metabolic rate has been thoroughly confirmed in insects (Chown & Nicholson 2004) and spiders (Anderson 1970; Moulder & Reichle 1972; Moeur & Eriksen 1972; Seymour & Vinegar 1972; Humphreys 1975; Shillington 2005). Most spider studies have used animals acclimated to a particular temperature. I quantified temperature impacts on SMR of adult female crab spiders, *Misumenoides formosipes* and *Mecaphesa asperata*, acclimated to naturally fluctuating field conditions. Both spiders are diurnally active ambush predators that hunt on flowers, and temperatures of their floral microhabitats can exceed ambient temperature (T_a) by 10° C or more (Schmalhofer 1996). Consequently, *M. asperata* and *M. formosipes* may experience widely varying temperature over the course of a day. Previous work has shown that the two species respond differently to temperature: *M. formosipes* hunts well from 15–40° C, but experiences a sharp decline in hunting performance at 10° C, whereas *M. asperata* hunts equally well from 10–40° C (Schmalhofer 1996; Schmalhofer & Casey 1999); *M. formosipes* also tolerates and prefers higher temperature than *M. asperata* (Schmalhofer 1999). I predicted that SMR would increase with increasing temperature in both species and that Q_{10}s would reflect the pattern shown by spider hunting performance (i.e., consistent Q_{10}s over temperature intervals where hunting performance was consistent, higher Q_{10}s over temperature intervals where hunting performance declined).

Although the impact of body size on spider metabolic rate has been well established (Greenstone & Bennett 1980; Anderson & Prestwich 1982; Anderson 1996), the complicating factor of reproductive condition has not been addressed. In female spiders, reproductive state strongly influences mass. Hence, a spider's reproductive condition could potentially affect metabolic rate. Kotiaho (1998) proposed that metabolic rate differs with reproductive condition among female spiders, and Walker & Irwin (2006) suggested that reproductive females would have higher metabolic rates than non-reproductive females. These hypotheses have not been tested. In this study, I quantified SMR of adult female *M. formosipes* in various states of hunger (fed or starved) and reproductive condition (virgin or mated). I predicted that although whole animal SMR would increase with increasing spider mass, mass-specific SMR would be equivalent among *M. formosipes* of differing hunger-reproductive condition (null model).

Many studies have generated mass-metabolism equations for particular spider species or families, for spiders in general, and for broader taxonomic categories, such as arthropods and ectotherms. I combined the mass-metabolism data obtained for *M. asperata* and *M. formosipes* with published data to generate a compilation data set, which I used to evaluate the mass-metabolism relationship of spiders in general. Although most studies have used adult female spiders to determine size-metabolism relationships, reproductive condition has not been explicitly considered. I compared SMR estimates, generated

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Table 1.—Field ambient temperature (T_a) preceding the measurement of crab spider SMR. I obtained temperature data (° C) from the Hutcheson Memorial Forest Research Center, Somerset County, New Jersey. I calculated average daily T_a from daily high and low T_a measurements. Average difference was based on the difference between a given day's high and low T_a (range: 6–24° C for both species). Maximum difference was the difference between the highest high T_a and lowest low T_a during a particular time period. Values in parentheses are ± 1SD.

Time frame	Average daily T_a	Average difference	Temperature range		Maximum difference
			Daily high	Daily low	
Spring 1994					
Collection to testing May 16–July 4 (50 days)	19.9 (4.8)	14.4 (4.3)	13.3–35.0	1.1–20.6	33.9
Two weeks prior to testing June 21–July 4 (14 days)	23.3 (1.8)	11.6 (4.0)	26.7–32.2	11.7–19.4	20.5
Summer 1994					
Collection to testing July 25–Sept. 18 (56 days)	20.4 (3.7)	13.7 (3.8)	21.1–32.2	3.9–20.6	28.3
Two weeks prior to testing Sept. 4–18 (14 days)	18.1 (3.3)	16.1 (2.8)	22.8–31.1	3.9–17.2	27.2

by published mass-metabolism equations and equations derived in the current study, with measured SMR values for crab spiders to assess the utility of the various equations in predicting SMR. I tested the null hypotheses that equation-generated estimates would not differ from measured SMR and that the equations would not differ from one another in their predictive ability.

This study is the first to examine the impacts of temperature on spiders acclimated to naturally fluctuating field conditions and to evaluate the joint influences of hunger, reproductive condition, and temperature on SMR. Results of this investigation will permit future estimations of foraging costs in field populations.

METHODS

Study animals.—*Misumenoides formosipes* and *M. asperata* are sit-and-wait predators that use enlarged, raptorial forelimbs, rather than a web, to capture prey. These spiders are widely distributed throughout North America (Gertsch 1939), semelparous, and have a lifespan of one year. Adults are seasonally separated: in central New Jersey, *M. asperata* matures in April–May, while *M. formosipes* matures in mid-August. I used only adult female spiders in this study and collected spiders from three field sites in Middlesex County and two field sites in Somerset County, New Jersey, USA. I did not consider population of origin as a factor in my analyses, although it is likely that experimental spiders represented two or three distinct populations for each species. Voucher specimens reside at the American Museum of Natural History, New York.

I kept spiders in small vials, plugged with moistened cotton balls, in a shaded, well-ventilated, outdoor enclosure. Consequently, spiders experienced field T_a, which varied over the course of a day and from time of collection to time of testing (Table 1). I fed spiders 2–3 flies (muscids and calliphorids; fly mass ≈ 25 mg) per week, which is comparable to the rate of prey capture in the field (Schmalhofer 2001). The amount of food in a spider's gut approaches zero after six days fasting (Nakamura 1972, 1987); in order to preclude variations in metabolic rate resulting from the absorption of food from the gut (Anderson 1970), I withheld food from "fed" spiders for seven days prior to measuring SMR. "Starved" *M. formosipes* fasted 25 days prior to testing, a time span that should have allowed metabolic rates to stabilize after any decline induced by starvation (Anderson 1974).

Experimental temperature range.—I tested spiders over an ecologically relevant temperature range: 10–40° C (*M. asperata* at 5° C intervals, *M. formosipes* at 10° C intervals). During May and June (i.e., when penultimate instar and adult *M. asperata* are active), daytime high T_a averages (mean ± SD) 25.1 ± 5.2° C, while nighttime low T_a averages 10.7 ± 5.2° C. Daytime high T_a from mid-July through mid-September (i.e., when penultimate-instar and adult *M. formosipes* are active) averages 29.4 ± 3.5° C, while nighttime low T_a averages 15.0 ± 4.6° C. (I determined averages using daily high/low temperature measurements taken at the Hutcheson Memorial Forest Research Center, Somerset County, New Jersey, from 1993 to 1995.) Compared to *M. asperata*, *M. formosipes* experiences an approximately 5° C upward shift in diurnal and nocturnal T_a. Because both *M. asperata* and *M. formosipes* may experience higher-than-ambient daytime temperatures due to the sun-exposed nature of their floral hunting sites, a temperature range of 10–40° C describes much of the thermal variation typically experienced by adult spiders in the field (Schmalhofer 1996).

Hunger and reproductive condition.—*Mecaphesa asperata* matures in early spring, and timing of maturation in this species is not as well-synchronized as it is in *M. formosipes*. The *M. asperata* I collected did not molt during their time in captivity, indicating that they were adults when collected. Consequently, I only examined temperature impacts on SMR in this species. The early work with *M. asperata* suggested, however, that it would be interesting to examine the impact of body size on SMR more thoroughly, and manipulating hunger state and reproductive condition provided a mechanism to generate a wide range of spider body sizes.

Controlling for hunger and reproductive condition of *M. formosipes* resulted from a combination of random and non-random assignment of treatments. Using a 2×2 design, I established four hunger-reproductive conditions of *M. for-*

mosipes: fed-mated, fed-virgin, starved-mated, and starved-virgin. I assigned spiders collected from the field as adults to the fed-mated category; spiders collected as juveniles I assigned to the fed-virgin, starved-virgin, and starved-mated categories. *Misumenoides formosipes* collected as adults were either clearly egg-heavy (spiders collected in September, $n = 2$) or did not appear obviously pregnant (spiders collected in mid-to-late August, $n = 2$). Although female crab spiders mate soon after reaching maturity, typically within 1–2 days (LeGrand & Morse 2000; Morse 2007), I provided adults collected in mid-to-late August with the opportunity to mate, just to be certain. In my experiments, I intended that fed-gravid spiders represent the higher end of the size (mass) spectrum that *M. formosipes* was capable of achieving. Mated spiders eating a normal field diet (which included large prey, such as honeybees and bumblebees) achieved much larger body mass that did mated spiders fed the captivity diet of muscid and calliphorid flies (Schmalhofer, pers. obs.). In order to maximize mass as much as possible, I marked the adults collected in mid-to-late August, released them back into the field, and recollected them in early September once they had achieved an "egg-heavy" appearance. I manipulated the reproductive condition of sub-adult females (spiders collected in late July and early-to-mid August, $n = 11$) by randomly assigning them to be mated or not once they underwent their final molt. Mass and SMR of starved-mated and starved-virgin *M. formosipes* did not differ (Mann-Whitney *U*-tests, P = NS in both cases), therefore I combined these spiders, and subsequent analyses dealt with only three categories: starved, fed-virgin, and fed-gravid. I used the term "gravid" to denote the extremely egg-heavy condition of fed-mated individuals.

Duration of captivity did not appear to affect the maturation schedule of *M. formosipes*. The spiders used in the present study were part of a much larger group of spiders ($n = 173$) collected for use in other experiments, and approximately half of these spiders underwent their final molt between the 15th and 25th of August. Spiders collected at different times (July 25–29, July 30–August 5, August 6–12) showed similar proportions (54–63%) of individuals molting during the August 15–25 period.

Metabolic rate measurement.—I determined SMR during daylight hours over a two-day period for each species. Spiders were resting, fasting (i.e., post-absorptive), and the test-range of temperatures (10–40° C) fell within the tolerance limits of both species (Schmalhofer 1999). Consequently, metabolic rate measurements satisfied the criteria for SMR (IUPS 2001). Although some spider species show temporal variation in oxygen consumption (Anderson 1970), I did not expect *M. asperata* and *M. formosipes* to do so because they hunt both diurnally and nocturnally (Schmalhofer 1996). SMR obtained for *M. formosipes* and *M. asperata* in the present study were comparable to the nocturnally measured SMR obtained by Anderson (1996) for *M. formosipes* and *Mecaphesa celer* (Hentz 1847), respectively.

A respirometer chamber consisted of a 60-cm^3 syringe with an attached three-way valve. Prior to spider placement, I pumped a syringe twice to flush the air inside. After introducing a spider, I expelled as much air as possible from the syringe (without squashing the spider – interior volume reduced to 3 cm^3), then drew room air into the syringe to a volume of 60 cm^3 and closed the valve. I collected control samples (empty syringes containing only a 60 cm^3 sample of room air) in the same manner. I placed spider and control syringes in a temperature box, where they remained for 2–5 h. I recorded time and barometric pressure both when spiders were placed in and removed from the temperature box.

I used an Amitek S-3A oxygen analyzer equipped with an N37 medical sensor to measure oxygen content of air samples. Both cells of the sensor had tygon tubes attached (≈ 2 m length, 0.32 cm inside diameter), and air drawn through each line passed through a separate desiccant (drierite) tube; I injected air samples into line 2 via a three-way stopcock. An R-2 flow controller (Amitek) maintained flow rate at 40 ml min^{-1} in each channel. To test an air sample, I closed the stopcock connected to line 2 and measured baseline delta (channel one minus channel two); I then drew a 40 cm^3 sample of air from a spider or control syringe into a sampling syringe, connected the stopcock on the sampling syringe with the stopcock on line 2, opened both stopcocks and injected the air sample into channel two of the oxygen analyzer. Injection of an air sample took less than 1 second and flushed the entire tygon tube of room air, replacing it with sample air. The large pressure transient disappeared within a few seconds, followed by a return to baseline. Delta max occurred about 1 min later and remained stable for approximately 1 min, then gradually returned to baseline as the sample washed out of the tube and room air replaced it. After injection of an air sample, it took approximately 3 min for the S-3A readout to peak and return to baseline. I tested air samples at 4–5 min intervals and interspersed measurement of spider samples with control samples. I calculated SMR as oxygen consumption ($\dot{V}_{O_2}$) in μl h^{-1} corrected to standard temperature and pressure dry (STPD) conditions using the equation of Bartholomew & Casey (1978). For STPD corrections, I used average barometric pressure based on barometric pressure when spiders were placed in and removed from the temperature box. I weighed spiders immediately prior to placement in the syringes.

Open system (flow through) respirometry with real-time measurement of O_2 consumption or CO_2 production has become the preferred method for measuring metabolic rate. The advantage of open system respirometry is the ability to factor out active periods, permitting more accurate measurement of SMR. Closed systems, such as the one used in my study, require the measurement of metabolic rate over prolonged intervals and may incorporate both active and inactive periods, leading to overestimation of metabolic rate (Lighton & Fielden 1995). In the case of spiders, however, closed and open system respirometry yield similar results (Lighton & Fielden 1995). Crab spiders in particular are extremely sedentary, negating the need to factor out periods of elevated metabolic rate caused by bouts of activity: once placed in a small container, *M. asperata* and *M. formosipes* quickly settle down, assuming the classic, stationary, crab spider hunting posture, and remain motionless for hours at a time.

I measured $\dot{V}_{O_2}$ for each spider at each test temperature. Because regression lines for *M. asperata* using data collected at 5° C intervals and 10° C intervals were nearly identical, I tested *M. formosipes* at 10° C intervals. For each species, I measured

metabolic rate near the end of the time frame in which adult female spiders were typically found in the field: *M. asperata*, early July; *M. formosipes*, mid-September.

Temperature and crab spider SMR.—I used linear regression to generate equations describing the relationship between temperature and mass-specific $\dot{V}_{O_2}$: I calculated regression equations for each individual, each species, and for each hunger-reproductive condition of *M. formosipes*. Using ANCOVA, I compared the mass-specific $\dot{V}_{O_2}$-temperature relationships shown by these crab spiders to one another and to published data for other spider species.

Temperature coefficients.—I calculated Q_{10}s for each species and for each *M. formosipes* hunger-reproductive condition at low temperature (10–20° C), mid-range temperature (20–30° C), and high temperature (30–40° C). Using Kruskal-Wallis tests, I compared Q_{10}s within a species across the experimental temperature range, and, within a given 10° C interval, I compared Q_{10}s among *M. formosipes* hunger-reproductive conditions. Where Kruskal-Wallis tests were significant, I made *a posteriori* pair-wise comparisons using Mann-Whitney *U*-tests.

Impacts of temperature, hunger, and reproductive condition on SMR of *M. formosipes*.—To assess joint impacts of temperature, hunger and reproductive condition on mass-specific $\dot{V}_{O_2}$ of *M. formosipes*, I used repeated measures ANOVA, followed by univariate ANOVAs to examine differences among spider conditions at a given temperature. Initially, I used live mass to calculate mass-specific $\dot{V}_{O_2}$. However, because lipids are not as metabolically active as proteins, and spider eggs are lipid-dense (Anderson 1978), I repeated these tests, adjusting mass and metabolic rate of fed spiders to remove the contribution of eggs/lipids. Female spiders accumulate yolk in eggs prior to copulation (Foelix 1996); therefore, I adjusted mass and SMR of fed-virgin as well as fed-gravid *M. formosipes*. For adjusted mass, I used mass measured just after spiders underwent their final molt, assuming that all mass gained between the final molt and the time I measured SMR was due to egg production and fat (yolk) accumulation. (For spiders collected as adults in mid-to-late August, mass at time of collection was used in place of mass at final molt. For spiders collected in September, mass at final molt was estimated based on the percentage of body mass gained between collection and testing of the August-collected adults.) I assumed that eggs and associated lipids had similar $\dot{V}_{O_2}$, and using data of Anderson (1978), I derived an average mass-specific $\dot{V}_{O_2}$ for spider eggs/lipids of 12.8 $\mu l\ g^{-1}\ h^{-1}$ at 15° C. I temperature-corrected egg/lipid mass-specific $\dot{V}_{O_2}$ using individual Q_{10}s for each spider, and subtracted $\dot{V}_{O_2}$ due to eggs/lipids from whole-animal $\dot{V}_{O_2}$ to obtain adjusted $\dot{V}_{O_2}$.

Estimating crab spider SMR from equations relating SMR to body size.—I applied equations relating SMR to live mass, drawn from the literature and derived in this study, to my experimental spiders. Using Mann-Whitney *U*-tests, I compared measured SMR to equation-generated SMR estimates for: 1) each of the three hunger-reproductive conditions of *M. formosipes* considered individually, 2) for *M. formosipes* considered collectively (pooling the three hunger-reproductive conditions together), and 3) for *M. asperata*. Most of the available literature data measured SMR in $\mu l\ O_2\ h^{-1}$ at 20° C. Where oxygen consumption was measured at a different temperature (i.e., Greenstone & Bennett 1982), I converted literature data to 20° C by assuming a Q_{10} of 2.5, as done by Lighton & Fielden (1995). Lighton & Fielden (1995) measured metabolic rate (based on CO_2 production) in μW at 25° C; for comparison, I used my crab spider data collected at 25° C (*M. asperata*), or estimated from individual spider regression equations (*M. formosipes*), and applied a conversion factor of 20.1 J per ml O_2, which assumes a respiratory quotient of 0.8 (Bartholomew 1981), to convert between $\mu l\ O_2\ h^{-1}$, $J\ h^{-1}$, and μW.

To compare the accuracy of the various equations in estimating crab spider SMR in general, I combined data for *M. formosipes* and *M. asperata* and determined the similarity between actual and estimated SMR. I calculated an index of similarity by dividing estimated SMR by measured SMR and used ANOVA to compare similarity scores among mass-metabolism equations.

Generalized spider mass-metabolism relationship.—To examine the general relationship between spider metabolism and live mass, I combined metabolic rates measured for *M. asperata* and *M. formosipes* at 20° C with published data. I used only data that met the criteria for SMR (i.e., spiders were rested and fasting) and selected protocols with three days of fasting as the minimum time period sufficient to ensure that spiders were post-absorptive. Nakamura (1987) showed that spider metabolic rate declines precipitously for the first 2–3 days post-feeding, but levels off by day 3–4, although the gut is not fully empty until approximately six days post-feeding. Data of Anderson (1970, 1996), Greenstone & Bennett (1980), Anderson & Prestwich (1982) and Shillington (2005) met the necessary criteria: these studies typically fasted spiders for 6–7 days; Anderson & Prestwich (1982) fasted spiders 3–7 days, but indicated that all spiders were post-absorptive. The resulting compilation data set comprised 117 data points (individual spiders or species averages) representing 54 species from 18 families. I analyzed the data using the traditional method of linear regression and a newer multiple regression technique described by Meehan (2006), based on Gillooly et al. (2001).

Statistical tests.—I tested all data, including ratios, and confirmed that the data satisfied assumptions of normality and homogeneity of variance: mass, $\dot{V}_{O_2}$, and mass-specific $\dot{V}_{O_2}$ required $\log_{10}$ transformation. Where sample sizes were small, I used nonparametric tests on raw data. I adjusted significance values as needed for multiple comparisons (Bonferroni correction).

RESULTS

Temperature and body size strongly affected crab spider SMR. Manipulation of hunger and reproductive condition successfully generated a wide range of body sizes in *M. formosipes*: while individuals assigned to the various hunger-reproductive conditions were of similar size just after their final molt (Kruskal-Wallis test: $H = 3.708$, $P = 0.1566$), size at time of testing differed significantly (Kruskal-Wallis test: $H = 12.375$, $P = 0.0021$) and varied over a six-fold range (Table 2). Comparison of initial mass and mass at time of testing indicated that eggs/lipids constituted 39% and 68% of the mass of fed-virgin and fed-gravid spiders, respectively.

Table 2.—Mass (mg) of *M. asperata* and *M. formosipes* used in the experiments. Size of experimental spiders is compared to that of recently matured conspecific females. Values for mass are means (± 1 SD). For fed-gravid *M. formosipes* (which were collected as adults), mass at time of collection was used in place of mass at final molt. x = times, in right-hand column.

Spider	*n*	Mass	Mass range	Size relative to newly matured adult
Present study				
Mecaphesa asperata	9	55.5 (10.9)	40.8–71.7	2 x
Misumenoides formosipes	15	98.4 (54.5)	29.7–187.8	2.2 x
At time of testing				
Starved	5	37.7 (8.0)	29.7–47.7	0.86 x
Fed-virgin	6	100.8 (17.1)	79.3–123.1	2.3 x
Fed-gravid	4	170.6 (14.6)	152.2–187.8	3.9 x
At final molt				
Starved	5	45.0 (11.3)		
Fed-virgin	6	60.7 (11.2)		
Fed-gravid	4	54.8 (10.1)		
Comparison data				
Mecaphesa asperata				
Newly matured	72	28.1 (10.1)	10.7–56.2	1 x
Pre-ovipositional	24	61.9 (13.1)	34.2–84.2	2.2 x
Misumenoides formosipes				
Newly matured	176	44.0 (14.7)	10.3–104.8	1 x
Pre-ovipositional	36	149.4 (68.5)	73.7–407.0	3.4 x

Temperature and crab spider SMR.—Temperature strongly affected crab spider SMR (Table 3). Mass-specific $\dot{V}_{O_2}$ of both *M. asperata* and *M. formosipes* increased with increasing temperature, and temperature accounted for > 80% of the variation in metabolic rate. ANCOVA indicated that SMR-temperature relationships of the two crab spider species were nearly identical: neither slopes (ANCOVA, species × temperature) nor intercepts (ANCOVA, species) of the regression lines differed. Comparison of the mass-specific $\dot{V}_{O_2}$-temperature relationships of *M. asperata* and *M. formosipes* with those published for other species (Table 4) revealed that although y-intercepts varied (ANCOVA, source, $F = 292.859$, $P < 0.0001$), slopes were equivalent (ANCOVA, source × temperature, $F=1.855$ $P = 0.1075$).

Temperature coefficients.—SMR of *M. asperata* displayed equivalent Q_{10}s across the experimental temperature range, while SMR of *M. formosipes* showed a significantly higher Q_{10} at low temperature than at mid-range temperature or high temperature (Table 5). Among the three hunger-reproductive conditions of *M. formosipes*, no clear pattern emerged other than that starved spiders tended to have higher Q_{10}s at the upper and lower ends of the experimental temperature range than did fed spiders.

Impacts of temperature, hunger, and reproductive condition on SMR of *M. formosipes*.—Hunger-reproductive condition and temperature significantly affected mass-specific $\dot{V}_{O_2}$ of *M. formosipes* (Table 6). When I used live mass to calculate mass-specific $\dot{V}_{O_2}$, I found that fed-gravid spiders typically had significantly lower mass-specific $\dot{V}_{O_2}$ at all temperatures except 10° C (Fig. 1A). When I removed the contributions of eggs/lipids to mass and SMR of fed spiders, I found that starved spiders generally had lower mass-specific SMR than fed

Table 3.—ANCOVA and linear regressions of temperature impacts on mass-specific SMR of *M. asperata* and *M. formosipes*. Temperature (T_a in the regression equation) was measured in ° C. Metabolic rate ($\dot{V}_{O_2}$ in the regression equation) was measured as oxygen consumption in $\mu l\ g^{-1}\ h^{-1}$ using live mass.

Test	*F*	*df*	r^2	*P*	regression equation
ANCOVA:					
Spider species	0.007	1		0.9348	
Temperature	541.023	1		< 0.0001	
Species x temperature	0.019	1		0.8913	
Linear Regression:					
Both species combined	592.293	1, 117	0.835	< 0.0001	$\log \dot{V}_{O_2} = 1.405 + 0.033\ T_a$
Mecaphesa asperata	253.352	1, 58	0.814	< 0.0001	$\log \dot{V}_{O_2} = 1.407 + 0.033\ T_a$
Misumenoides formosipes	292.455	1, 58	0.835	< 0.0001	$\log \dot{V}_{O_2} = 1.413 + 0.033\ T_a$
Starved	173.59	1, 18	0.906	< 0.0001	$\log \dot{V}_{O_2} = 1.322 + 0.038\ T_a$
Fed-virgin	162.62	1, 22	0.881	< 0.0001	$\log \dot{V}_{O_2} = 1.538 + 0.031\ T_a$
Fed-gravid	128.546	1, 14	0.902	< 0.0001	$\log \dot{V}_{O_2} = 1.338 + 0.030\ T_a$

Table 4.—Mass-specific SMR-temperature regression equations presented in the literature or derived from literature data. SMR ($\dot{V}_{O_2}$ in the regression equations) was measured as oxygen consumption in μl g^{-1} h^{-1} and was based on live spider mass. Temperature (T_a in the regression equations) was measured in ° C. Average value for the slopes (semi-log) of the SMR-temperature regressions, including those for *M. asperata* and *M. formosipes*, was 0.035 (SE = 0.002).

Literature source & spider	Regression equation	Derivation of regression equation
Moulder & Reichle (1972)		
thomisids, gnaphosids, lycosids	$\log \dot{V}_{O_2} = 1.696 + 0.032\ T_a$	Given in paper
Seymour & Vinegar (1973)		
Aphonopelma sp.	$\log \dot{V}_{O_2} = 1.065 + 0.029\ T_a$	Estimated from Fig. 2 data, 10–40° C
	$\log \dot{V}_{O_2} = 0.754 + 0.038\ T_a$	Estimated from Fig. 3 data, 20–40° C
Anderson (1970)		
Lycosa lenta	$\log \dot{V}_{O_2} = 1.087 + 0.042\ T_a$	Calculated from Table 5 data, 10–30° C
Phidippus regius	$\log \dot{V}_{O_2} = 1.155 + 0.040\ T_a$	Calculated from Table 5 data, 10–30° C
Filistata hibernalis	$\log \dot{V}_{O_2} = 0.738 + 0.048\ T_a$	Calculated from Table 5 data, 10–30° C
Moeur & Eriksen (1972)		
Lycosa carolinensis		
January spiders	$\log \dot{V}_{O_2} = 1.595 + 0.026\ T_a$	Calculated from Table 1 data: 23.5° C, 29° C, 35° C, 39° C, 45° C
June spiders	$\log \dot{V}_{O_2} = 1.491 + 0.025\ T_a$	Calculated from Table 1 data: 29° C, 35° C, 39° C, 45° C

spiders (Fig. 1B); whole animal $\dot{V}_{O_2}$ showed a similar pattern (Fig. 1C). Mass of starved spiders was significantly lower than adjusted mass of fed spiders (Kruskal-Wallis test: $H = 8.312$, $P = 0.0152$), averaging 65% of that of fed spiders. Whole animal $\dot{V}_{O_2}$ of starved spiders averaged 37% that of fed spiders (comparison of raw data, $\dot{V}_{O_2}$ of fed spiders adjusted to remove egg/lipid contributions).

Estimating crab spider SMR from equations relating SMR to body size.—With the notable exception of Hemmingsen's equation, the various mass-metabolism equations predicted crab spider SMR reasonably well (Fig. 2). The significant ANOVA ($F = 10.723$, $df = 8$, $P < 0.0001$) was driven by Hemmingsen's equation, which consistently over-estimated crab spider SMR. No differences in average predictive ability occurred among the other equations.

The various equations did not predict measured SMR of individual species, or hunger-reproductive conditions of *M. formosipes*, equally well (Table 7). Measured SMR of *M. asperata* was lower than all estimates, often significantly so. In contrast, estimates were generally equivalent to measured SMR of *M. formosipes* (considered collectively). Of the hunger-reproductive conditions of *M. formosipes*, the various equations usually predicted SMR of starved spiders quite well, but tended to under-estimate SMR of fed-virgin spiders and over-estimate SMR of fed-gravid spiders.

Generalized spider mass-metabolism relationship.—Both linear regression and multiple regression generated mass-scaling exponents of approximately 0.67: linear regression, $F = 706.546$, $df = 1,117$, $r^2 = 0.86$, $P < 0.0001$, $\log \dot{V}_{O_2} = -0.132 + 0.654\ (\log M)$ or $\dot{V}_{O_2} = 0.738\ M^{0.654}$, where $\dot{V}_{O_2}$ is oxygen consumption (μl h^{-1}) and M is mass (mg); multiple regression, $F = 364.97$, $df = 2,114$, $r^2 = 0.865$, $P_{total} < 0.0001$, $P_{intercept} = 0.0013$, $P_{mass} < 0.0001$, $P_{temp} = 0.0005$, $ln\ \dot{V}_{O_2} = 48.421 + 0.667\ (ln\ M) - 1.334\ (1/kT)$, where $\dot{V}_{O_2}$ is oxygen consumption (J h^{-1}), M is mass (mg), k is Boltzmann's constant (0.0000862), and T is temperature (K). (Note: in the latter portion of the multiple regression equation, units cancel out because 1.334 has units of eV and Boltzmann's constant has units of eV K^{-1}.) SMR of *M.*

Table 5.—Temperature coefficients (Q_{10}s) of *M. asperata* and *M. formosipes* across the experimental temperature range. Values presented are means (± 1 SD). I used Kruskal-Wallis tests to compare Q_{10} values within a given species. I also compared Q_{10} values within a given temperature interval among *M. formosipes* hunger-reproductive conditions (adjusted $\alpha \leq 0.0167$, Bonferonni correction for multiple comparisons). If Kruskal-Wallis tests were significant, I used Mann-Whitney U-tests to make pair-wise comparisons: values with different letters are significantly different ($P \leq 0.05$).

Spider	Temperature interval			Kruskal-Wallis *P*
	10–20° C	20–30° C	30–40° C	
Mecaphesa asperata Q_{10}	2.35 (0.84)	2.04 (0.94)	2.40 (0.96)	0.4498
Misumenoides formosipes Q_{10}	3.90 (0.72)[a]	1.69 (0.40)[b]	1.75 (0.52)[b]	< 0.0001
M. formosipes categories:	Starved Q_{10}	Fed-virgin Q_{10}	Fed-gravid Q_{10}	
Temperature interval				
10–20° C	4.32 (0.55)	4.03 (0.73)	3.20 (0.34)	0.0463
20–30° C	1.72 (0.55)	1.55 (0.27)	1.87 (0.37)	0.2563
30–40° C	2.22 (0.52)	1.61 (0.36)	1.38 (0.31)	0.0435

Table 6.—Repeated measures ANOVA examining impacts of temperature (°C) and spider condition on mass-specific SMR [log ($\mu l\ O_2\ g^{-1}\ h^{-1}$)] of *M. formosipes*. Tests were run on data calculated using live spider mass and on data in which mass and SMR of fed spiders were adjusted to remove contributions of eggs/lipids. Associated univariate ANOVAs comparing mass-specific SMR among spider conditions at a given temperature are also provided. For univariate ANOVAs, a significant difference occurs at $\alpha \leq 0.0125$ (Bonferroni correction for multiple comparisons).

Test and effect	*df*	*F*	*P*
Live mass			
Repeated measures ANOVA			
Spider condition	2	20.011	< 0.0001
Temperature	3	541.291	< 0.0001
Interaction	6	3.736	0.0054
Univariate ANOVAs			
10° C	2	4.401	0.0368
20° C	2	12.946	0.0010
30° C	2	9.572	0.0033
40° C	2	32.896	< 0.0001
Adjusted mass & SMR			
Repeated measures ANOVA			
Spider condition	2	48.921	< 0.0001
Temperature	3	541.799	< 0.0001
Interaction	6	3.727	0.0055
Univariate ANOVAs			
10° C	2	22.921	< 0.0001
20° C	2	18.758	0.0002
30° C	2	28.586	< 0.0001
40° C	2	7.625	0.0073

asperata and *M. formosipes* at 20° C fit well within the general scatter of literature data (Fig. 3).

DISCUSSION

Temperature strongly affected crab spider SMR. As predicted, mass-specific $\dot{V}_{O_2}$ increased with increasing temperature, and Q_{10}s reflected temperature impacts on crab spider hunting performance. Whole-animal $\dot{V}_{O_2}$ increased with increasing body size, as expected, but contrary to my prediction, mass-specific $\dot{V}_{O_2}$ of *M. formosipes* differed with hunger or reproductive condition, and the precise impact depended on the nature of the mass-specific $\dot{V}_{O_2}$ calculation. Spider SMR scaled as 2/3 of live body mass, and most mass-metabolism equations generated reasonable estimates of (collective) crab spider SMR; however, estimates were not as accurate for fed spiders (mated or virgin) as they were for starved spiders. These results point to the need for caution when evaluating spider SMR: accurate assessment requires knowledge of spider hunger and reproductive condition.

Temperature and crab spider SMR.—Given that spiders are strict ectotherms (Pulz 1987), a strong impact of temperature on SMR of *M. asperata* and *M. formosipes* was expected. Nor was it surprising that neither degree of hunger nor reproductive condition affected the general nature of the temperature-metabolism relationship. Many studies have shown that metabolic rate increases with increasing temperature in spiders and other terrestrial arthropods (Anderson 1970; Moulder & Reichle 1972; Seymour & Vinegar 1973; Humphreys 1975; Lighton et al. 2001; Meehan 2006). The slope of the regression line relating mass-specific $\dot{V}_{O_2}$ to temperature is remarkably consistent among spider species, suggesting a relatively high degree of conformity among spiders in their response to temperature.

Temperature coefficients.—Q_{10}s describe the effects of temperature changes on the rates of physiological processes or biochemical reactions (Hochachka & Somero 1984; Wilmer et al. 2005), and metabolic rates typically have Q_{10}s of 2–3 (Wilmer et al. 2005). As predicted, Q_{10}s for crab spider SMR correlated with temperature impacts on spider hunting performance. Q_{10}s for SMR of *M. asperata* varied between 2.0–2.4 across the experimental temperature range, suggesting that *M. asperata* is active and functions normally between 10–40° C. In contrast to *M. asperata*, SMR of *M. formosipes* showed a significantly higher Q_{10} at low temperature than at moderate temperature or high temperature. High Q_{10} at low temperature is a common response in ectotherms (Hoffman 1985) and has been proposed as a means of conserving energy during thermally unfavorable periods (e.g. Aleksiuk 1976); as temperature increases, a greater-than-normal increase in metabolic rate allows normal activity to resume quickly. The dramatic increase in $\dot{V}_{O_2}$ of *M. formosipes* occurring between 10–20° C suggests that *M. formosipes* is not normally active at 10° C. The difference between the two crab spider species in Q_{10} at low temperature also correlates with seasonal differences in temperature during the species' adult and penultimate instars, with *M. formosipes* experiencing temperatures averaging 5° C higher than those experienced by *M. asperata*.

Impacts of temperature, hunger, and reproductive condition on SMR of *M. formosipes*.—Manipulation of hunger and reproductive condition produced spiders that differed significantly in mass at the time of testing, although they had been of similar initial mass. Neither hunger nor reproductive condition changed the general nature of the temperature-$\dot{V}_{O_2}$ relationship in *M. formosipes*; metabolic rate increased with increasing temperature, and regression slopes were similar among all three conditions. Hunger or reproductive condition did, however, have a significant impact on mass-specific $\dot{V}_{O_2}$, and the nature of the effect depended on whether I used live mass or whether I removed the contribution of eggs/lipids when calculating mass-specific $\dot{V}_{O_2}$.

Using live mass, temperature interacted with spider condition to affect $\dot{V}_{O_2}$; mass-specific SMR of *M. formosipes* did not differ among conditions at 10° C, but at all other temperatures, fed-gravid spiders had lower mass-specific $\dot{V}_{O_2}$ than fed-virgin or starved spiders. The similarity among conditions at 10° C could reflect a general suppression of metabolic rate at low temperature in *M. formosipes*. At higher temperatures, the lower mass-specific $\dot{V}_{O_2}$ of fed-gravid spiders resulted from the large contribution of egg mass to total body mass. Anderson (1978) found that free-living spiders had metabolic rates almost an order of magnitude higher than those of developing eggs. Eggs held within a female's body prior to oviposition should likewise be relatively metabolically inert. Because fats are less metabolically active than proteins, and spider eggs contain a large amount of lipid (Anderson 1978), the more egg-heavy the spider, the greater the proportionate contribution of lipid-dense tissue to overall body mass, and, consequently, the lower the mass-specific $\dot{V}_{O_2}$

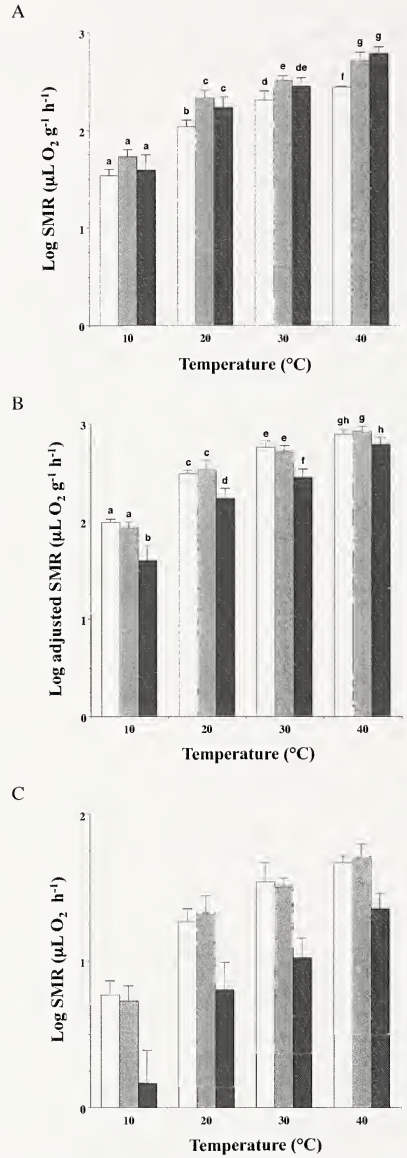

Figure 1.—Average SMR of *M. formosipes* hunger-reproductive conditions across the experimental temperature range. Within a test temperature, values with different letters are significantly different at $\alpha \leq 0.0125$ (Bonferroni correction for multiple comparisons) using a Bonferroni-Dunn post-hoc test. Comparisons were made only among hunger-reproductive conditions within a given temperature, not across temperatures. Error bars = 1 SD. Symbols: □ = fed-gravid, ■ = fed-virgin, ■ = starved. A. Mass-specific SMR calculated using live mass. At 30° C, starved and fed-gravid spiders were nearly significantly different ($P = 0.0127$). B. Mass-specific SMR calculated using adjusted mass and SMR for fed spiders (contributions of eggs/lipids removed). At 40° C, starved and fed-gravid spiders were nearly significantly different ($P = 0.0194$). C. Whole-animal SMR provided for comparison; SMR of fed spiders has not been adjusted to remove contributions of eggs/lipids.

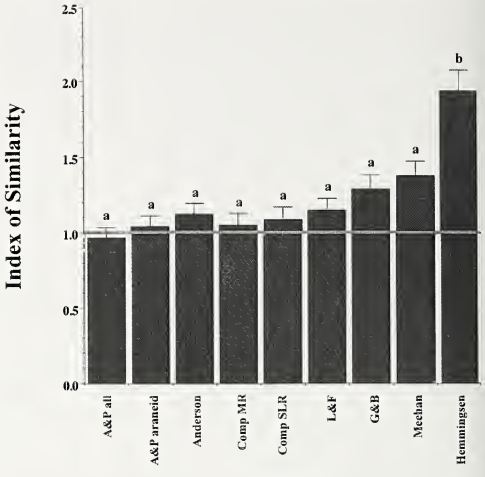

Figure 2.—Average similarity between measured crab spider SMR (*M. asperata* and *M. formosipes* combined) and SMR estimated using mass-metabolism equations. I calculated the index of similarity as estimated SMR divided by measured SMR. The closer to one an equation's similarity score, the better it predicted crab spider SMR. Values with different letters are significantly different at $\alpha \leq 0.05$ (Scheffé post-hoc test). Error bars = 1 SE. A&P all = Anderson & Prestwich (1982) all spiders; A&P araneid = Anderson & Prestwich (1982) araneids only; Anderson = Anderson (1996) thomisids; Comp MR = compilation data set, multiple regression (this study), spiders; Comp SLR = compilation data set, linear regression (this study), spiders; L&F = Lighton & Fielden (1995) arthropods (ants, beetles, spiders); G&B = Greenstone & Bennett (1980) spiders; Meehan = Meehan (2006) arthropods (orobatid mites, springtails, spiders); Hemmingsen = Hemmingsen (1960) ectotherms.

compared to non-gravid spiders whose body composition is proportionately less lipid-dense. Approximately two-thirds of the mass of fed-gravid *M. formosipes* consisted of eggs. This is typical of flower-dwelling crab spiders: other studies have found that eggs constitute more than 60% of female pre-oviposition weight (Fritz & Morse 1985; Beck & Connor 1992; Schmalhofer unpubl. data).

Mass-specific $\dot{V}_{O_2}$ does not totally eliminate the influence of body size on metabolic rate because mass and metabolism share an allometric relationship (Packard & Boardman 1999). ANCOVA on whole-animal $\dot{V}_{O_2}$, with mass as the covariate,

Table 7.—Comparison of measured SMR of *M. asperata* and *M. formosipes* with estimated SMR based on various mass-metabolism equations: Lighton & Fielden (1995), arthropods, $\dot{V}_{O_2} = 906M^{0.825}$; Anderson (1996), *M. formosipes*, $\dot{V}_{O_2} = 0.62M^{0.7}$, *M. celer*, $\dot{V}_{O_2} = 0.52M^{0.71}$; Anderson & Prestwich (1982), all spiders, $\dot{V}_{O_2} = 0.33M^{0.8}$, araneids, $\dot{V}_{O_2} = 0.18M^{0.96}$; Greenstone & Bennett (1980), spiders, $\dot{V}_{O_2} = 0.736M^{0.71}$ at 22° C, $\dot{V}_{O_2} = 0.698M^{0.71}$ at 20° C; Meehan (2006), arthropods, $ln(\dot{V}_{O_2}) = 18.42 + 0.77\ [ln(M)] - 0.58$ (l/kT); Hemmingson (1960), ectotherms, $\dot{V}_{O_2} = 0.82M^{0.75}$; compilation SLR (this study), spiders, $\dot{V}_{O_2} = 0.738M^{0.654}$; compilation MR (this study), spiders, $ln(\dot{V}_{O_2}) = 47.354 + 0.677\ [ln(M)] - 1.308$ (l/kT). Anderson's (1996) equations for *M. formosipes* and *M. celer* were compared to *M. formosipes* and *M. asperata*, respectively. For Meehan (2006) and the compilation multiple regression, $\dot{V}_{O_2}$ was calculated in J h^{-1}, but converted back to µl h^{-1} for this table. Comparisons with Lighton & Fielden (1995) were made in µW at 25° C. Mann-Whitney *U*-tests were used to compare measured values with equation-generated estimates: a significant difference occurs at $\alpha \leq 0.0056$ (Bonferroni correction for multiple comparisons). Values presented are means (±1SD). †$P \leq 0.05$, *$P \leq 0.0056$.

Source	*Mecaphesa asperata*	*Misumenoides formosipes*			
		All	Starved	Fed-virgin	Fed-gravid
Measured SMR					
µl h^{-1} at 20° C	7.7 (1.7)	16.1 (7.9)	6.9 (3.3)	21.9 (5.2)	19.0 (3.8)
µW at 25° C	64.9 (26.8)	130.7 (53.2)	64.1 (17.1)	167.0 (24.7)	159.4 (25.6)
Estimated SMR					
Lighton & Fielden (1995)	83.7 (12.7)	130.8 (61.6)	60.5 (10.6)	136.2 (18.0†)	210.6 (14.9)†
Anderson (1996)	9.0 (1.2)	14.9 (6.1)	7.8 (1.2)	15.6 (1.8)	22.6 (1.4)
Anderson & Prestwich (1982)					
All spiders	8.2 (1.2)	12.7 (5.8)	6.0 (1.0)	13.2 (1.7)	20.1 (1.4)
Araneids	8.6 (1.5)	14.7 (7.8)	5.9 (1.2)	15.1 (2.3)†	25.0 (2.1)†
Greenstone & Bennett (1980)	12.8 (1.7) *	18.5 (7.7)	9.7 (1.5)	19.4 (2.2)	28.3 (1.7)†
Meehan (2006)	11.8 (1.7) *	17.8 (7.9)	8.7 (1.4)	18.6 (2.3)	28.0 (1.8)†
Hemmingsen (1960)	16.7 (2.3) *	24.9 (10.8)†	12.4 (2.0)†	26.0 (3.1)	38.7 (2.5)†
Compilation SLR	9.5 (1.2) †	13.4 (5.1)	7.4 (1.0)	14.0 (1.5)†	19.9 (1.1)
Compilation MR	9.2 (1.1)†	13.0 (5.1)	7.1 (1.0)	13.4 (1.5)†	19.4 (1.1)

can resolve this issue (Packard & Boardman 1988, 1999). However, an underlying assumption of using ANCOVA is that all mass behaves similarly with respect to impacts on metabolic rate. This was not the case for these spiders, since a large fraction of the mass of fed spiders was a composed of metabolically inactive tissue that contributed little to total metabolism. Adjusting mass and metabolism to exclude the influence of non-metabolizing tissue before examining mass-specific SMR was a more appropriate, although not perfect, solution.

Removing the estimated contribution of eggs/lipids to mass and SMR of fed spiders revealed that starved *M. formosipes* had lower mass-specific $\dot{V}_{O_2}$ than fed spiders. Reductions in metabolic rate attributed to starvation by many authors actually reflect attainment of a post-absorptive state in which energy is no longer being used for digestion and assimilation (Nakamura 1987). True suppression of metabolic rate as a consequence of prolonged starvation, as reported by Anderson (1974), has seldom been shown. I found that the percent reduction in $\dot{V}_{O_2}$ of starved *M. formosipes* was comparable to that measured by Anderson (1974) for starved *Kukulcania hibernalis* (Hentz 1842) (as *Filistata hibernalis*) and *Hogna lenta* (Hentz 1844) (as *Lycosa lenta*): at 20° C, mass-specific SMR was reduced by 32% in *H. lenta*, 40% in *K. hibernalis*, and 47% in *M. formosipes*. (Because Anderson's study involved non-fat, non-reproductive spiders, my results were not directly comparable until I adjusted for egg/lipid contributions to mass and $\dot{V}_{O_2}$.) It is possible that the reduction in SMR seen in starved *M. formosipes* was a result of decreased mass rather than physiologic changes associated with prolonged starvation. Starved spiders lost 15% of body mass during the fasting period and had lower mass than fed spiders, even after removal of egg/lipid mass from the latter, so mass was not "equalized" among treatment groups. However, it seems likely that the reduced metabolic rate observed in starved *M. formosipes* was an effect of starvation beyond loss of mass: differences between fed and starved spiders in mass and $\dot{V}_{O_2}$ were disproportionate (mass and $\dot{V}_{O_2}$ of starved spiders averaged 65% and 37%, respectively, of that of fed spiders), whereas differences between mass and $\dot{V}_{O_2}$ of fed-gravid and fed-virgin spiders were proportionate. Hence, true starvation-induced suppression of metabolic rate, as seen in long-lived, iteroparous species (Anderson 1974), also appears to occur in the short-lived, semelparous *M. formosipes*. It may be that starvation-induced suppression of metabolism is a general phenomenon in spiders; further studies with other species are needed.

Elevation of metabolic rate as a consequence of reproductive condition has been shown in various ectothermic species, such as rattlesnakes (Beaupre & Duvall 1998) and lizards (Angilleta & Sears 2000). Walker & Irwin (2006) predicted that spiders would behave similarly, with reproductive females having higher mass-specific metabolic rates than non-reproductive females. My data did not support this hypothesis: mass-specific $\dot{V}_{O_2}$ of fed-gravid *M. formosipes* was equivalent to or lower than that of fed-virgin *M. formosipes*. *Misumenoides formosipes* is not unique in this respect: differences in metabolic rates of reproductive and non-reproductive mites have also been found to be explicable on the basis of body mass (Young & Block 1980). Why spiders and mites differ from vertebrate ectotherms in this regard is not clear.

Estimating crab spider SMR from equations relating SMR to body size.—With the notable exception of Hemmingsen's equation, the various mass-metabolism equations were statistically indistinguishable from one another and, on average, provided reasonably accurate estimates of crab spider SMR,

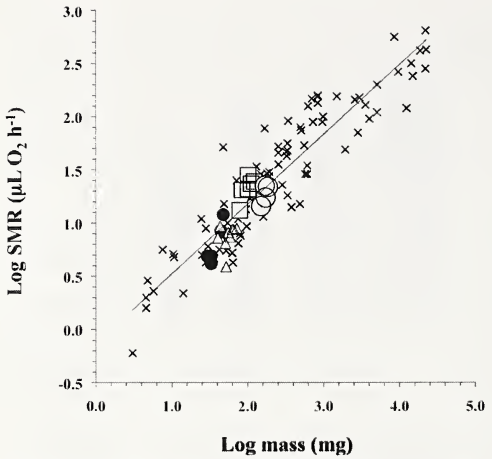

Figure 3.—Relationship between spider SMR and live mass at 20° C. Each data point represents an individual spider or a species average. I determined the regression line using linear regression: log $\dot{V}_{O_2}$ = −0.132 + 0.654 (log M). Literature sources: Greenstone & Bennett (1982), 47 individuals; Anderson (1970), 6 species averages and 15 individuals; Anderson (1996), 12 species averages; Anderson & Prestwich (1982), 12 species averages; Shillington (2005), 1 species average. Data for Anderson & Prestwich were estimated from Anderson & Prestwich (1982), Figure 1; the resulting mass-metabolism equation based on these estimates ($\dot{V}_{O_2}$ = 0.321 $M^{0.802}$) was nearly identical to the equation derived by Anderson & Prestwich ($\dot{V}_{O_2}$ = 0.33 $M^{0.8}$). Symbols: ×= literature data, ●= *Misumenoides* starved, □= *Misumenoides* fed-virgin, ○= *Misumenoides* fed-gravid, △= *Mecaphesa*.

based on live mass. Most of the equations generated estimates of crab spider metabolic rate that were somewhat higher than actual measured values. Hemmingsen's equation, however, greatly over-estimated crab spider SMR, yielding estimates that were nearly double actual values and significantly larger than other estimates. Similar results when comparing spider metabolic rates with estimates based on Hemmingsen's equation are common (e.g. Anderson 1970; Greenstone & Bennett 1980; Anderson & Prestwich 1982; Strazny & Perry 1987). Hemmingsen (1960) has frequently been cited for comparative purposes due to its comprehensive nature (Anderson 1970) and because it expanded the study of metabolic mass scaling to include ectotherms (Dodds et al. 2001; White & Seymour 2005). Widespread use of Hemmingsen's equation as a yardstick for comparison led to the general conclusion that spiders have exceptionally low metabolic rates for arthropods of their size (Anderson 1970; Greenstone & Bennett 1980; Anderson & Prestwich 1982; Strazny & Perry 1987). The utility and validity of Hemmingsen's equation have come into question (Lighton & Fielden 1995; Dodds et al. 2001), however, and spider metabolic rates have been found not to differ from those of non-spider arthropods (Lighton & Fielden 1995; Meehan 2006).

When considering how well the various mass-metabolism equations predicted SMR of a particular crab spider species or hunger-reproductive condition of *M. formosipes*, I obtained mixed results. Over-estimates of SMR generated for fed-gravid *M. formosipes* generally balanced out under-estimates calculated for fed-virgin spiders. Combined with the accuracy of estimates for starved spiders, the equations typically yielded fairly accurate estimates of metabolic rate for *M. formosipes* in total. SMR of *M. asperata*, in contrast, was not as well predicted. I did not manipulate reproductive condition in this species, but body mass suggested that most *M. asperata* were gravid, and, like fed-gravid *M. formosipes*, actual SMR was lower than estimated SMR. To circumvent reproductive complications in evaluating metabolic rate, one needs to exclude the contribution of eggs and associated lipids to total body mass and to express metabolic rate in terms of adjusted "egg/lipid free" mass. In the present study, once I removed egg/lipid mass I found that starved spiders, not fed-gravid spiders, had the lowest mass-specific $\dot{V}_{O_2}$.

The technique of excluding metabolically inactive tissue from metabolic rate measurements has yielded interesting results in other contexts. Djawden et al. (1997) found that stressed lineages of fruit flies had lower mass-specific SMR than non-stressed control lineages and suggested that differential accumulation of lipids and carbohydrates was the cause; they also suggested that fundamental changes in metabolic rate were best detected by expressing metabolic rate in a manner that did not include the mass of non-metabolizing material, and when they accounted for non-metabolizing sources, the differences in metabolic rates between stressed and non-stressed lineages disappeared.

Generalized spider mass-metabolism relationship.—One of the most contentious issues in environmental physiology involves the determination of what constitutes a "characteristic" metabolic rate for an animal of a given size (Chown & Nicholson 2004). The relationship between mass and metabolism is generally described by the allometric equation

$$V = aM^b,$$

which may also be written as

$$\log V = \log a + b(\log M),$$

where V is metabolic rate, M is body mass, and a and b are the intercept and slope, respectively, of the mass-metabolism regression. The value of b is of particular interest. The original null model, first proposed in the 1800s and based on simple dimensional analysis, hypothesizing that b = 0.67, was supplanted in the early 1900s by empirical studies indicating that b = 0.75 (see review by White & Seymour 2005). Aspects of some of the early work widely cited in support of a 3/4 scaling exponent (e.g. Kleiber 1932; Brody 1945; Hemmingsen 1960) have been questioned (e.g. Lighton & Fielden 1995; Dodds et al. 2001; White & Seymour 2005). Consequently, the value of b, which had been accepted as 0.75 for decades, has been subject to re-evaluation, with some authors supporting b = 0.67 (e.g. Dodds et al. 2001; White & Seymour 2005), others maintaining that b = 0.75 (e.g. West et al. 1997; Gillooly et al. 2001; West & Brown 2005), and still others arguing in favor of an entirely different exponent for particular groups of animals. For instance, Lighton et al. (2001) suggest that the mass-scaling exponent for non-tick, non-scorpion arthropods is 0.856. In the present study, I found that SMR of spiders scales as approximately 2/3 of live body mass, regardless of method

used: linear regression, b = 0.654 (SE = 0.025); multiple regression, b = 0.677 (SE = 0.025). If SMR values for individuals of a given species within a study were averaged in order to reduce the over-representation of particular species in the compilation data set, sample size of the compilation data set was reduced to 60, but b still approximated 2/3: linear regression, b = 0.668 (SE = 0.035); multiple regression, b = 0.678 (SE = 0.036).

Conclusions.—Temperature exerted a strong impact on crab spider metabolic rate, and temperature impacts on *M. formosipes* and *M. asperata* were comparable to those found for other spider species. Prolonged starvation resulted in a decrease in SMR of *M. formosipes* beyond that which normally occurs as spiders attain a post-absorptive state. Mass-specific $\dot{V}_{O_2}$ of fed-gravid *M. formosipes* was lower than or equivalent to that of fed-virgin *M. formosipes* (depending on how mass-specific SMR was calculated). The low metabolic rate of egg-heavy females, when live mass was used to calculate mass-specific $\dot{V}_{O_2}$, was an artifact of the large contribution of lipid-rich, metabolically-inactive eggs to female mass. Because this effect is expected to be universal among spiders, caution should be exercised when interpreting the results of spider metabolic rate measurements, and reproductive condition of adult female spiders should be taken into account. Ideally, in experiments investigating how factors that affect body size ultimately affect metabolic rate, pre-treatment and post-treatment metabolic rates should be determined so that treatment effects can be compared against a true baseline measure. A control group fed a diet designed to maintain constant body mass should also be used to account for potential impacts of time (aging) on the animals.

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Do cannibalism and kin recognition occur in just-emerged crab spiderlings?

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Abstract. Most spiders are aggressive, socially intolerant predators; however, broods develop inside a common site and thus should benefit from restraining aggression at this time and until they disperse. I tested single and mixed-brood groups of *Misumena vatia* (Clerck 1757) (Thomisidae) spiderlings that had just emerged from their nests to determine whether they cannibalized other young under crowded conditions comparable to the immediate area of their nests, and if so, whether they distinguished between sibs and non-sibs. Young *M. vatia* provide an interesting test case, since some broods remain in close contact for a short period of time after emerging from their nests. Mortality remained low over one month in provisioned young under crowded conditions, and no cannibalism occurred in these individuals. Cannibalism remained low in most broods of unprovisioned young, even though most of them eventually starved over this time. Just-emerged spiderlings placed in the field for three days and then run similarly also showed initially low tendencies toward cannibalism. However, larger free-ranging spiderlings that overlapped in size with provisioned spiderlings in the study cannibalized freely when confined similarly to the other spiderlings in this study. During this period the spiderlings showed no clear evidence of distinguishing between sibs and non-sibs.

Keywords: Crowding, *Misumena vatia*, starvation, Thomisidae

Cannibalism, the ingestion of all or part of a conspecific (Pfennig 1997), occurs naturally in a wide range of animals (Pfennig 1997; Osawa 2002; Hvam et al. 2005). However, its impact within populations typically has elicited only limited attention (Fox 1975; Polis 1981; Elgar & Crespi 1992), and it remains relatively poorly understood (Wilder & Rypstra 2010. Yet, cannibalism may play an important role in regulating both even-aged and size-structured populations whose large individuals prey on small ones (Polis & McCormick 1987; Fagan & Odell 1996). Cannibalism may even occur within a cohort (Klingenberg & Spence 1996; Wagner & Wise 1996). For instance, Wagner & Wise (1996) found that intracohort cannibalism in a litter-dwelling wolf spider population played the major role in engendering density-dependent control, and Hvam et al. (2005) obtained similar results with another wolf spider.

Most spiders are highly predatory, socially intolerant animals and in many instances will kill one another if confined (Foelix 1996a; Wise 2006), behavior consistent with the normally solitary existence of the vast majority of species. A critical stage thus takes place immediately after they emerge from their natal sites, when spiderlings of diverse species remain in sibling groups prior to dispersing. Relatively few spiders provide parental care (Foelix 1996a), which might decrease cannibalism, although social and subsocial spiders remain together and may discriminate between kin and non-kin (Evans 1999; Bilde & Lubin 2001; Beavis et al. 2007). Since they start their independent lives with a large yolk sac spiderlings have little initial need to cannibalize, though they may readily take prey at this time. Studies examining whether spiderlings of solitary species routinely attack each other at this time have reported differing results. In one such study Roberts et al. (2003) found that second-instar wolf spiders *Hogna hellulo* (Walckenaer 1837) exhibited both kin recognition and a reluctance to cannibalize kin, in contrast to other wolf spiders (Wagner & Wise 1996; Hvam et al. 2005).

A reluctance to cannibalize could be general or specific to the brood (Hvam et al. 2005). Recognition of one's offspring or sibs may assume considerable selective significance in directing predation away from closely related individuals. Although widely distributed among animals, kin recognition is seldom reported among solitary arthropods (Hepper 1991; Faraji et al. 2000). Some apparent examples of kin recognition may simply reflect a response to general similarity, making discrimination a more appropriate term (Hvam et al. 2005; Wise 2006). For instance, a group of siblings may be of similar size, but differ in size from members of other conspecific broods, predisposing the larger to cannibalize the smaller (Chapman et al. 1999).

Similar size and the consequent substantial danger of attempting cannibalism may inhibit this behavior within a brood without evoking kin recognition (Chapman et al. 1999; Wise 2006). In some species cannibalism only occurs as the animals approach starvation (Evans 1999; Bilde & Lubin 2001). However, Roberts et al. (2003) found no increase in cannibalism among individuals of different size or in starved *H. hellulo* sibs. Differences may also vary with sex and stage (Agarwala & Dixon 1993; Joseph et al. 1999; Osawa 2002). Individuals that remain together (social insects and social spiders) usually exhibit restraint, as do certain other arthropods without rapid, highly developed dispersal (e.g., phytoseiid mites: Faraji et al. 2000; Schausberger & Croft 2004).

Several of the studies on cannibalism and kin recognition have taken place in the laboratory under crowded conditions that the participants would seldom if ever experience for more than a brief period under natural settings (e.g., Wagner & Wise 1996; Rickers & Scheu 2005; Dobler & Kölliker 2010). However, they take on considerable interest because they simulate brief, but potentially important, stages of the life cycle and may thus illuminate conditions that occur naturally in the field.

The crab spider *Misumena vatia* (Thomisidae), an aggressive solitary species, provides an interesting opportunity to address questions of cannibalism and kin recognition early in life. Individuals remain within their natal nests until part way through their second instar and normally disperse soon after,

but occasionally remain together immediately outside their nest for a day or more before dispersing (Morse 2007). Thus, they occur temporarily in extremely crowded situations, both inside and outside of their nest. These conditions thus resemble those of crowded laboratory experiments and provide the basis for the experiments presented here. Specifically, I ask, 1) do recently emerged spiderlings cannibalize at this stage, 2) does food (or its absence) affect these results, and 3) do recently emerged spiderlings exhibit kin recognition? Preferentially cannibalizing non-kin would provide evidence for Question 3.

METHODS

The species.—Female *Misumena* lay a single large clutch of 75–300+ eggs in a nest constructed by turning under the distal end of an elliptical leaf and tightly binding the resulting domicile with silk (illustrated in Morse 1985, 2007). Although mothers guard their nests for a considerable period, they provide no active protection for their young after the latter emerge from the nest (Morse 1985), in contrast to spiders that shelter their offspring for several days (e.g., wolf spiders: Rovner et al. 1973). The young emerge from their nests about 26 days after egg-laying, having by then undergone one molt (Morse 1985). Shortly before leaving their nests the young second instars begin to make holes through the silk in the nest that allow them access to the exterior and routinely occupy these exits or even venture outside. Usually they abandon their nests within a few days after construction of the nest holes (Morse 1987, but see Morse 2011). Occasionally they congregate for up to a day or more immediately outside a nest hole, but usually they disperse within a day after final emergence, either by walking or on lines to nearby hunting sites, often goldenrod (*Solidago* spp.) inflorescences, or by ballooning greater distances if they do not quickly find hunting sites (Morse 1993). Spiderlings' normally rapid dispersal suggests their vulnerability at this time, and cannibalism represents one such possible danger.

However, unequivocally demonstrating cannibalism presents a possible problem. *Misumena* do not masticate their prey, and I could not find wounds on the victims. Crab spiders make microscopic holes, only about 50 μm × 50 μm in rectangular wounds made by adult female *Xysticus cristatus* (Clerck 1757), which quickly fill with rapidly drying hemolymph upon withdrawal of the chelicerae (Foelix 1996b). Holes made by *Misumena* spiderlings will make much smaller holes than mature *Xysticus*.

Spiderling *Misumena* typically only take live prey (D.H. Morse, pers. obs.), such that observations of spiderlings feeding on conspecifics probably represent cannibalism events. Further, early-instar *Misumena* feed much longer on conspecifics than on similar-sized *Drosophila melanogaster*, collapsing the conspecifics' abdomens, so that they become concave (rather than convex), a condition seen in each instance of cannibalism or apparent cannibalism (feeding upon conspecifics), including two observations of successful attacks (D.H. Morse pers. obs.). The long feeding times also heighten the probability of observing apparent cannibalism in the process of monitoring, maintaining and observing the spiderlings. I obtained minimum feeding times for seven instances of apparent cannibalism. The apparent cannibals had already begun to feed on their victims in each of these observations, so the actual times necessarily exceeded those recorded.

Experiments.—I used members of 31 broods of spiderlings as the primary subjects in this study. All came from experimentally mated parents, using virgin females to ensure full sibship of brood members.

In order to test for cannibalism, the effect of food upon the propensity to cannibalize, and kin recognition, I used 14 pairs of broods. For each pair, I set up two treatments with 10 siblings, with or without food, and two treatments of mixed broods, five spiderlings each, with or without food. In addition to these 14 complete designs (28 broods), I included three incomplete designs (three broods) where appropriate. Since broods emerged sequentially over a few weeks, I assigned pairs on the basis of which broods emerged at nearly the same time.

All individuals of each brood had emerged from their nests within the preceding two days and had not fed before I set up the experimental groups, using individuals selected haphazardly from the broods. I marked each individual with either red or blue powdered micronite dye to identify it to brood, the colors randomly designated by brood. Previous studies indicate that the dye does not affect their behavior (Morse 1993, 2000a. I housed all the groups in cylindrical 7-dram vials (5 cm tall, 3-cm diameter) at natural day lengths and provided them with a small (2 cm^2) square of paper toweling, moistened every other day. This enclosure provided them with a space comparable in size to the congregating sites immediately outside their natal nests (Morse 2007). Individuals in the provisioned groups received one *Drosophila melanogaster* per test member every other day. Second and third instars grow rapidly on this diet (Morse 2000b). I inspected all groups daily, as well as at other random times, for deaths or molts. On average this work required approximately an hour per day in each year I ran these experiments (2001, 2002, 2007, 2008, 2010), during which I simultaneously made observations on the spiderlings.

I weighed individuals from 12 of these broods at the start of the study, but did not subsequently weigh them in order to avoid further observer effects. For the same reason I did not again mark any individuals that had molted or whose color had become so faint that it hindered recognition. In most instances this strategy confined brood identity of the mixed groups to the second instar; many individuals molted once or occasionally twice during the study.

I also ran a control to test the possible effect of maintaining multiple individuals in a confined space, rearing 20 spiderlings individually (one per vial) from each of 17 broods for one month in similar vials and providing them with one *Drosophila* every other day, similarly to the experimentals. I then compared their month-long survival with that of the experimental groups. None of these individuals came from the afore-mentioned 31 broods.

In addition to the above-mentioned groups of spiderlings tested, I ran three additional groups of spiderlings in 2010 in order to gather additional insight into the role of cannibalism. I elaborate upon them in the following three paragraphs and refer to them in quotation marks in order to minimize confusion.

I observed two pairs of these experimental broods, set up as described above, intensively ("intensively-observed group"),

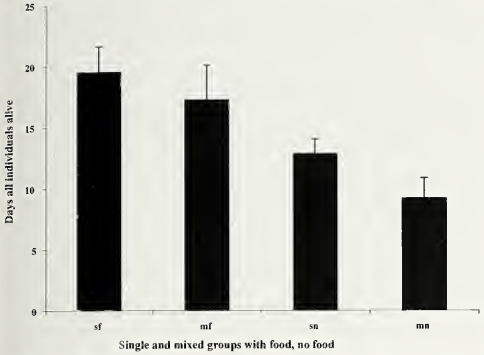

Figure 1.—Number of days that all individuals of one- and two-brood groups survived with and without food, mean + SE. Abbreviations: sf = single brood with food, mf = two-brood group with food, sn = single brood group without food, mn = two-brood group without food.

Figure 2.—Survivorship of *Misumena vatia* spiderlings in one- and two-brood groups with and without food over a month after emergence from their nests, mean + SE. Symbols as in Figure 1.

an extra hour or more per day, in addition to the time involved in maintenance. I thereby accumulated a large number of spider-hours, since all of these groupings (12 vials) could be observed simultaneously.

I also released six entire color-marked broods (three pairs) into the field on goldenrod *Solidago canadensis*. Three days later I collected 15 individuals of each brood ("field-experienced group") and established them in 7-dram vials, as in the previous experiment: 10 individuals each of both broods and five individuals of both broods in a third set. I also watched these broods approximately one hour each day over a 30-day period. All but five of the 90 individuals captured for this experiment exceeded the mean mass of their broods when released (0.78 ± 0.02 vs. 0.48 ± 0.01 mg). Thus, most had probably captured one or two prey over this time and were not in a starved condition. When collected in the field, none of the individuals were spaced as densely as those in their nests or in the 7-dram vials. Since I wished to concentrate on the conditions most likely to elicit cannibalism, I did not establish sets of provisioned individuals in either this or the following (next paragraph) manipulation.

I also collected larger spiderlings ("large group") of unknown parentage from goldenrod and established seven sets of six individuals each, matched for size. I lowered numbers of individuals per 7-dram vial to six in light of their relatively large size. These individuals ranged from 1.19 to 5.50 mg and probably had been exposed to field conditions for one to three weeks. I maintained these spiderlings for 15 days.

One might question the effect of the confined conditions to which I exposed the spiderlings. However, the volume of the vials resembles their exposure immediately before emerging from their nest and the numbers that accumulate on the under surface of their nest immediately after emergence (Morse 2007). Thus, the main effect of confining the newly emerged spiderlings was to preclude dispersal. Although members of more than one brood would seldom mix at a dispersal site, early instars of different broods often accumulate at rich hunting sites soon after, not infrequently in high densities (Morse 1993).

Analysis.—I tested comparisons between broods with two-way ANOVAs or *t*-tests for the difference between two means. I used *G*-tests of independence or goodness of fit for 2 × 2 comparisons and a binomial test for a one-sample comparison. All tests are two-tailed, and all measures of variance are means ± 1 SE.

RESULTS

Survival.—A majority of the provisioned spiderlings survived for the entire 30-day experimental period (Fig. 1). Unprovisioned spiderlings lived for varying periods, but members of several broods died within a week of the start of the experiments (Fig. 1). Overall, the model comparing provisioned and unprovisioned individuals was significant ($F_{3,92} = 6.10$, $P < 0.001$). Provisioned and unprovisioned individuals differed highly significantly in survival time ($F_1 = 13.78$, $P < 0.001$). Single-brood groups survived marginally longer than two-brood groups ($F_1 = 3.57$, $P = 0.062$), but the interaction term was not significant ($F_1 = 0.02$, $P = 0.88$).

The same pattern occurred in the number of single-brood and two-brood groups of individuals alive at the end of a one-month run (Fig. 2), with a highly significant overall model ($F_{3,92} = 124.74$, $P < 0.0001$). A majority of provisioned individuals survived for a month, but very few unprovisioned individuals survived that long ($F_1 = 340.88$, $P < 0.0001$), and again the numbers from the one-brood group marginally exceeded those from the two-brood group ($F_1 = 3.26$, $P = 0.074$). Again, the interaction term was not significant ($F_1 = 0.02$, $P = 0.74$).

Survival of the separated spiderlings to one month (16.9 ± 0.52 of 17 broods = 84.5%) significantly exceeded that of the one-brood groups (72%: Fig. 2) ($t_{30} = 2.42$, $P = 0.022$), largely the consequence of the uncharacteristically low survival in two of the one-brood sets. (A non-parametric Mann-Whitney *U* test yielded a similar result: $P = 0.028$).

Initial mass did not affect survival in single-brood groups with food ($t_{15} = -0.64$, $P = 0.53$ for days that all individuals survived; $t_{15} = -0.42$, $P = 0.68$ for the number of individuals surviving one month). Neither did it significantly affect survival of those without food ($t_{15} = -0.94$, $P = 0.36$ for days that all individuals survived; $t_{15} = -1.85$, $P = 0.086$ for the number surviving one month), although a trend occurred toward large individuals surviving longer than small ones. I did not test two-brood groups in this way because after a molt I could not identify them to brood.

Cannibalism.—I observed only two probable instances of cannibalism among the provisioned spiderlings, both involving the deaths of males that had molted into the antepenultimate stage (Instar 4), at which point they differ markedly from the females. Both females (from different broods) fed on male sibs on Day 28. The spiderlings' tendency to take only live prey suggests that the females had killed their male sibs.

Five unprovisioned spiderlings lived to the end of these 30-day experiments, over twice the mean survival period (Fig. 2), probably by feeding on other individuals. As the sole remaining individuals, they had no other resources. Two came from single-brood groups and three from two-brood groups. Two of the latter survivors probably fed on fellow brood members and one on a member of the other brood. One of these five individuals weighed more after 30 days than at the beginning of the run.

I observed nine instances of probable cannibalism in the set of four "intensively-observed" broods, all spiderlings feeding on other individuals or fresh corpses found with conspicuously shrunken (concave) abdomens, the typical condition of conspecifics after being fed upon by spiderlings. Other spiderlings that had recently died did not have conspicuously concave abdomens. With one early exception, all these instances of apparent cannibalism in the "intensely observed" broods occurred only after two weeks or more, by the time that considerable numbers of unprovisioned spiderlings began to starve. All nine of these individuals came from the unprovisioned group ($P = 0.004$, binomial test), seven of them from the 40 individuals in the single-brood vials, not significantly different from the two out of 20 individuals in the mixed-brood vials ($G = 0.62$, $P > 0.3$, G-test). One of the two mixed-brood losses involved individuals from the same brood, but I could not identify the brood of the other one. Five of the nine apparent cannibalism events came from just one of the six vials of unprovisioned spiderlings (a single-brood vial), suggesting a predisposition toward cannibalism in one of the broods, though this relationship did not differ statistically from that in the other vials ($G_I = 1.56$, $P > 0.2$, G-test). However, one individual probably made most of these kills. It weighed 1.01 mg after 18 days, well over twice the mean mass of its brood at emergence from their nest (0.45 mg).

I observed two successful cannibalistic attacks by the six broods of "field-experienced" spiderlings, both eventually resulting in corpses with collapsed (concave) abdomens. I recorded 18 instances of cannibalism or apparent cannibalism from these six broods, not significantly different from the nine instances in the four intensively-observed broods of the preceding test ($G = 0.19$, $P > 0.5$), though the latter group was unusual in terms of its high apparent frequency of cannibalism. However, apparent cannibalism in the "field-experienced" spiderlings significantly exceeded that of the entire set of 31 broods used in the main set of experiments ($G = 12.50$, $P < 0.001$).

The "field-experienced" group tended to commence cannibalizing more quickly after the initiation of the experiment than the "intensively-observed" group, even though almost all had fed previously, judging from their increase in mass since release. Six of 18 events took place before 14 days, vs. one of nine in the naïve group ($G = 2.80$, $0.1 > P > 0.05$). Nine of the 22 individuals from the "field-experienced" group that survived for 30 days weighed more than the mean mass at Day 1 (0.78 mg), consistent with cannibalism. Three of the 18 events took place between broods vs. 15 within broods, a trend toward favoring sib cannibalism ($G = 3.09$, $0.1 > P > 0.05$). Of the three instances in the mixed broods, one occurred within-brood, one between-brood, and the other undetermined.

In contrast to the other groups, the "large" spiderlings experienced high apparent cannibalism from the start, even prior to the time at which I provided *Drosophila* to any groups of provisioned spiderlings. They cannibalized 19 of the 42 individuals within the first two days, evenly across the seven vials, and the number surviving had declined to seven by the end of Day 15, all fatalities apparently resulting from cannibalism. Mortality of these "large" spiderlings significantly exceeded that of both the "intensely observed" group ($G = 18.47$, 37.91 at two and 15 days, respectively) and the "field-experienced" group ($G = 97.14$, 23.28 at two and 15 days, respectively) ($P < 0.001$ in each instance).

Cannibals fed on their victims for a long period. I obtained minimum feeding times for seven of these cannibalization events in the "intensely observed" and "field-experienced" groups, which averaged over five and one-half hours (331 ± 64.4 min). Actual times probably considerably exceeded this figure, because all individuals had already begun feeding when first found.

Kin recognition.—The experiments provided no clear evidence of kin recognition, as recognized by differential survival or cannibalism rates in the mixed-brood experiments presented in the preceding sections. A few observations do provide possible anecdotal evidence for kin recognition. All five individuals of one brood in a mixed brood vial died on the second day, a pattern not repeated elsewhere. Since these individuals all came from one brood, and no other cohort of sibs died at the same time, cannibalism seems plausible. The trend for cannibalizing sibs in the "field-experienced" broods suggested a preference for sibs, though no such pattern emerged elsewhere, making the evidence in support of kin recognition, at most, equivocal.

Prey capture.—Provisioned spiderlings used in these experiments captured prey from the start of these experiments, each day collectively killing all of the flies presented them. Although the observational regime did not permit me to establish whether each individual captured a *Drosophila* on the first day, the ability of all individuals of some provisioned groups to survive to the end of the experiments, combined with the relatively rapid mortality of most unprovisioned groups, indicated that most of the spiders captured prey. Some individuals fed on a fly in tandem (not quantified), typically from the opposite ends of the victim. Failure to attack other

spiderlings was thus not related to a generalized reluctance to attack under these confined conditions.

DISCUSSION

Survivorship of provisioned single-brood and two-brood groups did not differ significantly over their first month, either in time to first mortality or mean survival time, though a weak trend occurred for single-brood groups to exceed two-brood groups. Although more solitary controls survived for a month than in provisioned groups, the modest differences between them could represent a stress factor associated with crowding, rather than cannibalism (Dobler & Kölliker 2010). A likely exception among the provisioned individuals, the demise of two precocious males, could result from the discrete changes occurring in some males at their last molt in the experiment (striping of legs, etc.: Hu & Morse 2004). These males would normally not experience cannibalism at this point, since they would not have remained in close contact with their female sibs. This putative cannibalism probably did not result from a behavioral change by the males, because they do not differ in activity from other immatures at this time and exhibit no signs of sexual activity (Sullivan and Morse 2004). However, the likely fate of those males resembles the differential treatment accorded sex and stage by various ladybird beetles (Agarwala & Dixon 1993; Joseph et al. 1999; Osawa 2002).

The unprovisioned spiders living in groups suggest that cannibalism is relatively uncommon in most, though not all, newly emerged *Misumena* broods, with the majority of them dying of apparent starvation. Although the simultaneous demise of all five members of a brood in a mixed group seems most likely attributable to cannibalism, it probably did not result from impending starvation, which facilitates cannibalism in some species (Evans 1999; Bilde & Lubin 2001) and likely accounted for most of the cannibalism of unprovisioned individuals recorded in this study.

If cannibalism occurred frequently, I should have recorded more potential examples among the 31 broods of just-emerged spiderlings. Although the observational regime did not permit continual surveillance, the spiderlings feed on prey (Erickson & Morse 1997; Morse 1999), especially conspecifics (this paper), for long periods, so that I would likely have regularly observed such events, had they frequently occurred. Observations of spiderlings feeding on other spiderlings likely represent cannibalism, since the spiderlings do not regularly scavenge dead organisms (Morse 2007). Dobler & Kölliker (2010) have, however, noted that all studies of this sort record very few actual observations of cannibalism, even if it is likely prevalent. Only constant surveillance will quantify this potential factor definitively. In fact, my only two observations of spiderlings successfully attacking and killing conspecifics occurred during extended observation periods. The larger number of apparent cannibalism events in the set of four "intensively-observed" broods probably results from the characteristics of these individuals, rather than a difference in procedure. Instances of one spiderling feeding on another are conspicuous and unlikely to be missed. Other results (Morse 2011) indicate considerable differences among broods in the propensity to cannibalize.

The reluctance to cannibalize even held in the unprovisioned "field-experienced" broods, although cannibalism commenced marginally sooner than in the comparison group of four "intensively-observed" broods and significantly sooner than in the just-emerged spiderlings. Still, no cannibalism occurred before the sixth day, thereby demonstrating a continuing reluctance to cannibalize either sibs or non-sibs.

The behavior of the "field-experienced" group differed strikingly from that of the randomly captured "large" spiderlings, whose numbers nearly halved over the first two days. These results suggest that a separation of more than three days is required to remove completely the inhibition to cannibalize. Although the "large" group of spiderlings cannibalized freely, provisioned spiderlings showed no tendency to cannibalize during the experimental period (30 days), over which time they overlapped with the "large" field-captured spiderlings in both mass and probable age.

Thus, the "field-experienced" group (in the field for three days) showed a possible reduced inhibition to cannibalize, and the "large" spiderlings, in the field for an estimated one to three weeks, showed no apparent inhibition to cannibalize. These results suggest that in most instances inhibition to cannibalize lasts for a few days, considerably longer than the spiderlings normally remain together, and that it declines over time until it disappears, as in the "large" spiderlings tested.

The low frequency of apparent cannibalism in the first half of the month-long observation period is consistent with other studies in which equivalent' participant size decreases cannibalistic tendencies (Chapman et al. 1999). The wolf spider *Pardosa agrestis* (Westring 1861) only cannibalizes victims half or less than half its size (Samu et al. 1999). However, some species do regularly cannibalize similar-sized conspecifics (Klingenberg & Spence 1996; Wagner & Wise 1996).

Clearly, factors other than size play a role in deterring cannibalism in these spiderlings, because both *Drosophila melanogaster* and the spiderlings' frequently abundant prey in the field, a small dance fly (Empididae) (Morse 1993, 2000a), are similar in size to the young spiderlings (Morse 2000b), though totally different in appearance. The spiderlings readily attack the flies immediately after emerging from their nests and they also attack *Drosophila* in the laboratory at this time (Morse 2000a).

In most instances the spiderlings appeared to treat sibs and non-sib conspecifics similarly. Though these data do not eliminate the possibility of kin recognition, the majority of these results strongly suggests that they typically interact similarly with sibs and non-sibs.

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Notes on the genus *Mesobuthus* (Scorpiones: Buthidae) in China, with description of a new species

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Abstract. *Mesobuthus karshius* new species from the southern region of Xinjiang, China, is described. Nine species and subspecies of the genus *Mesobuthus* Vachon 1950 from China are recorded, and diagnoses of *M. eupeus mongolicus* (Birula 1911), *M. eupeus thersites* (C.L. Koch 1839) and *M. martensii martensii* (Karsch 1879) are provided. In addition, *M. caucasicus przewalskii* (Birula 1897), *M. caucasicus intermedius* (Birula 1897), *M. eupeus mongolicus* (Birula 1911), *M. karshius* sp. nov. and *M. martensii martensii* (Karsch 1879) are illustrated, and a key to the Chinese *Mesobuthus* is also provided.

Keywords: New species, taxonomy, morphology, *Mesobuthus karshius*

The genus *Mesobuthus* Vachon 1950 currently includes 13 species (Fet & Lowe 2000; Gantenbein *et al.* 2000; Lourenço *et al.* 2005; Kovařík 2007; Sun & Zhu 2010; Sun et al. 2010), including one new species reported here. It is one of the most widely distributed genera of the family Buthidae, with species from the Balkans, Anatolian Peninsula, Iran, throughout Asia to China, Korea, and Japan. The composition of this large, predominantly Asian, genus has not been very clear until now, mainly because of its plentiful subspecies (Fet & Lowe 2000), especially in Iran and Afghanistan. The most useful publications involving *Mesobuthus* are old keys and reviews by Birula (1897, 1900, 1904, 1905, 1911, 1917), and the only recent revisions and keys for the genus focus on India (Tikader & Bastawade 1983) and Afghanistan (Vachon 1958).

The first species of *Mesobuthus* described from China was *M. martensii* by Karsch (1879), originally described as *Buthus martensii*. After the description of *M. martensii*, two other taxa, *M. caucasicus przewalskii* (Birula 1897) and *M. eupeus mongolicus* (Birula 1911) were described by Birula (1897, 1911) in the genus *Buthus* as *B. caucasicus przewalskii* and *B. eupeus mongolicus*. Moreover, Birula (1904) also described a new subspecies, *M. martensii hainanensis*, based on a single specimen of unknown sex from Hainan Island, as *B. confucius hainanensis*. More recently, *M. eupeus thersites* (C.L. Koch 1839) and *M. caucasicus intermedius* (Birula 1897) have also been recorded from China (Fet 1994; Fet & Lowe 2000). Lourenço *et al.* (2005) described the fourth species of this genus from China, *M. songi*, based on old preserved specimens from the northern piedmont of the Himalayas, Xizang (Tibet). This species has been found to belong to *Hottentotta* Birula 1908 (Sun et al. 2010). Here we provide the results of the first comprehensive investigation of all six *Mesobuthus* species from China (as well as six subspecies), as well as detailed illustrations of four previously established subspecies (*M. caucasicus przewalskii*, *M. caucasicus intermedius*, *M. eupeus mongolicus* and *M. martensii martensii*) and the description of a new species discovered from the Karshi (Kashgar) District, Xinjiang Uygur Autonomous Region, China.

METHODS

We examined and measured specimens under a Leica M165c stereomicroscope with an ocular micrometer. To produce illustrations, we used a Leica M165c stereomicroscope with a drawing tube. All measurements follow Stahnke (1970) and are given in millimeters (mm), except for the chela, in which we follow Vachon (1952). Trichobothrial notations follow Vachon (1974) and morphological terminology mostly follows Hjelle (1990). Specimens used in this taxonomic work come from the Museum of Hebei University, Baoding (MHBU) and the American Museum of Natural History, New York (AMNH).

TAXONOMY

Family Buthidae C.L. Koch 1837
Genus *Mesobuthus* Vachon 1950

Mesobuthus Vachon 1950:152; Vachon 1952:324; Vachon 1958:141; Stahnke 1972:133; Tikader & Bastawade 1983:186; Kovařík 1998:114; Fet & Braunwalder 2000:15–16, fig. 1; Fet et al. 2000:287–288; Fet & Lowe 2000:169; Karataş & Karataş 2001:297; Teruel 2002:75; Ganbentein et al. 2003:412, 417; Karataş & Karataş 2003:1; Soleglad & Fet 2003a:9, 12, 20, 26, table 2; Soleglad & Fet 2003b:12, 13, 19, 21, 53, 66, 68, 78, 88, 91, figs. 4, 15, 78, tables 3, 4, 9; Qi et al. 2004:137; Teruel et al. 2004:2, 5; Zhu et al. 2004:112; Fet et al. 2005:3, 7, 10, 12–13, 22, 29, table 1, fig. 23; Karataş 2005:1; Lourenço et al. 2005:2–3; Prendini & Wheeler 2005:451, 454, 481, table 3; Shi & Zhang 2005:474; Dupré 2007:7, 13, 17; Karataş 2007:1; Kovařík 2007:1–3, 8, 94; Shi et al. 2007:216; Kovařík 2009:24; Lourenço & Duhem 2009:38–39, 44, 48, 50; Sun & Zhu 2010:1; Sun et al. 2010:35.

Olivierus Farzanpay 1987:387 (synonymy by Ganbentein et al. 2003:417).

Type species.—*Androctonus eupeus* C.L. Koch 1839, by original designation.

Diagnosis.—See Vachon (1950); Sissom (1990) and Sun et al. (2010).

Distribution.—Species of *Mesobuthus* occur in Asia, the Balkan Peninsula and Caucasia.

Mesobuthus bolensis Sun, Zhu & Lourenço 2010
(Fig. 10)

Mesobuthus bolensis Sun et al. 2010:36–40, figs. 2, 3, 5–11, 14–18, 21, 22, table 1.

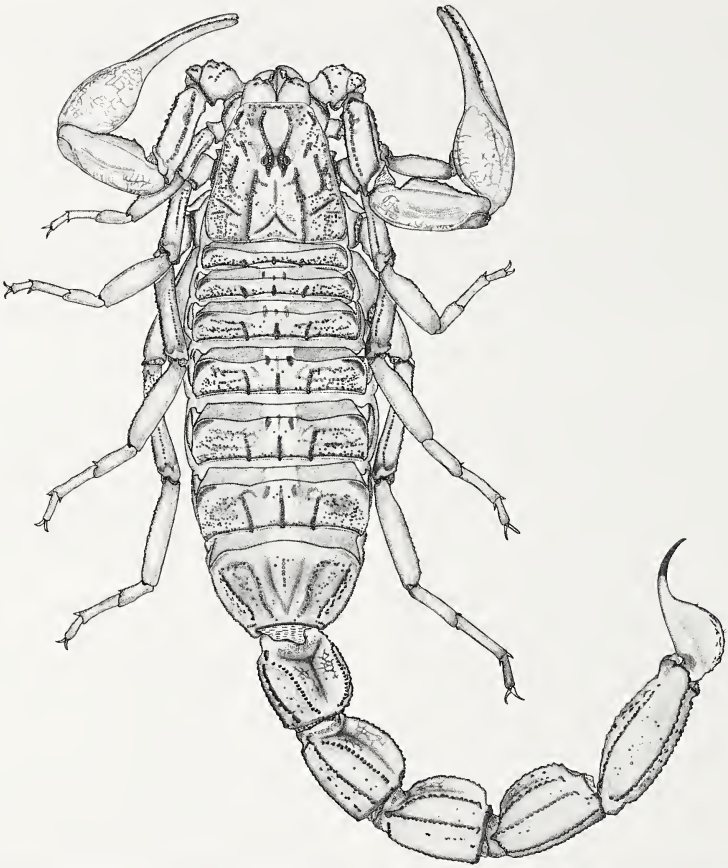

Figure 1.—*Mesobuthus caucasicus przewalskii* (Birula 1897), female from Tuokexun County, Xia Village (42°47′N, 88°40′E), dorsal view.

Material examined.—See Sun et al. (2010).

Diagnosis.—See Sun et al. (2010).

Distribution.—This species occurs in China (Xinjiang Uygur Autonomous Region).

Ecology.—See Sun et al. (2010).

Mesobuthus caucasicus przewalskii (Birula 1897)
(Figs. 1, 2, 10, Table 1)

Buthus caucasicus przewalskii Birula 1897:387.

Mesobuthus caucasicus przewalskii (Birula): Vachon 1958:148, fig. 31; Gantenbein et al. 2003:412; Qi et al. 2004:142; Shi & Zhang 2005:475; Sun & Zhu 2010:4–5, 7–8, figs. 3, 14–16.

Olivierus caucasicus przewalskii (Birula): Farzanpay 1987:156; Fet & Lowe 2000:192; Zhu et al. 2004:113.

Type specimens.—Type material not examined.

Material examined.—CHINA: Xinjiang Uygur Autonomous Region: Aksu City, 7 km SW of downtown area, near to West Bridge, 41°07′N, 80°11′E, 2 June 2009, D. Sun and Y.W. Zhao, 2 ♀, 2 ♂, 1 juvenile (MHBU); Artush City, area near to Arhu Town, 39°42′N, 76°09′E, 7 June 2009, D. Sun and Y.W. Zhao, 6 ♀, 3 ♂ (MHBU); Wuqia County, 39°44′N, 75°14′E, date and collector unknown, 2 ♀ (MHBU). Other material examined, see Sun et al. (2010).

Diagnosis.—See Sun et al. (2010).

Distribution.—*Mesobuthus caucasicus przewalskii* occurs in China (Xinjiang Uygur Autonomous Region), Tajikistan, Uzbekistan and Mongolia.

Ecology.—This subspecies is distributed from Mongolia, throughout Xinjiang, to Central Asia. In Xinjiang, most of specimens were collected in croplands (cotton or other) and vineyards, or around villages. In pure, natural environments

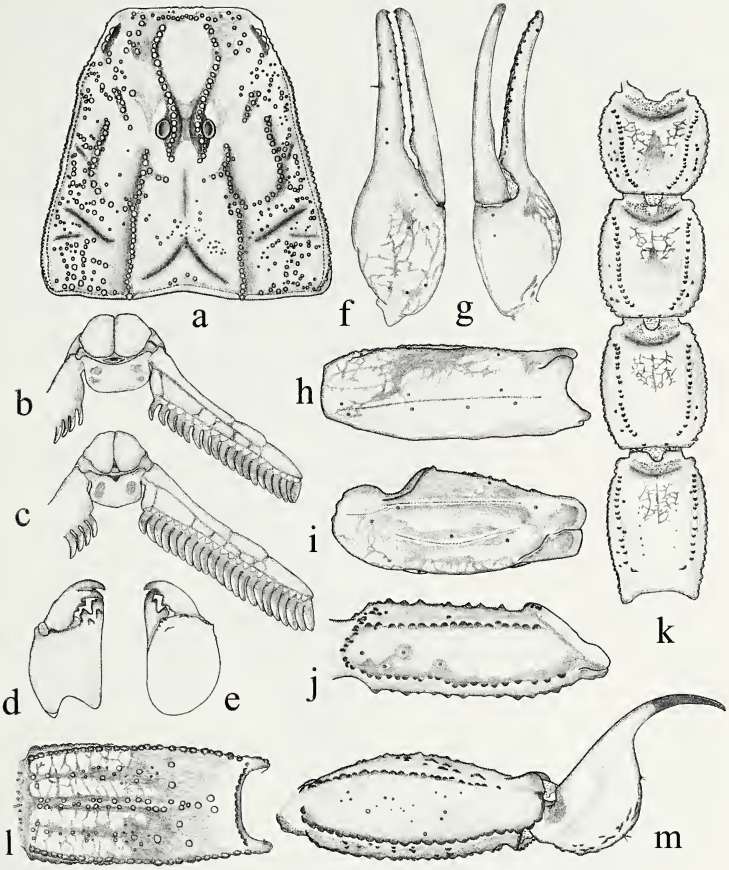

Figure 2.—Mesobuthus caucasicus przewalskii (Birula 1897), from Tuokexun County, Xia Village (42°47′N, 88°40′E): a, b, d–m: female; c: male. a. Carapace, dorsal aspect; b, c. Genital operculum and pectines, ventral aspect; d, e. Chelicera (d, ventral; e, dorsal); f, g. Chela (f, dorso-external; g, ventral); h, i. Patella (h, external; i, dorsal); j. Femur, dorsal aspect; k. Metasomal segment I–IV, dorsal aspect, showing the pigments; l. Metasomal segment V, ventral aspect; m. Metasomal segment V and telson, lateral aspect.

(the deserts or Gobi) the population density is quite low, probably mainly because of the lack of food and potential excessive water loss in high temperatures.

Mesobuthus caucasicus intermedius (Birula 1897)
(Figs. 3, 4, 10, Table 1)

Buthus caucasicus forma γ *intermedius* Birula 1897:387.
Buthus caucasicus intermedius (Birula): Birula 1900:368; Birula 1911:168; Pohl 1967:214.
Mesobuthus caucasicus intermedius (Birula): Vachon 1958:150, fig. 31; Kovařík 1997:49; Kovařík 1998:114; Qi et al. 2004:142; Shi & Zhang 2005:475; Sun & Zhu 2010:3–4, 7–8, figs. 2, 11–13.
Olivierus caucasicus intermedius (Birula): Farzanpay 1987:156; Fet & Lowe 2000:191; Zhu et al. 2004:113.

Type specimens.—Type material not examined.

Material examined.—CHINA: *Xinjiang Uygur Autonomous Region*: Yining City, 5 km E of downtown area, 43°55′N, 81°23′E, 14 August 2006, F. Zhang, H.Q. Ma and S.N. Liu, 1 ♀, 1 ♂; Bole City, 2 km SW of downtown area, south bank of canal, 44°52′N, 82°02′E, 31 July 2007, D. Sun and L. Zhang, 1 ♂. KAZAKHSTAN: see Sun et al. (2010).

Diagnosis.—See Sun et al. (2010). This subspecies is undoubtedly a close relative of *M. caucasicus przewalskii*, but it can be distinguished by the following features: 1)

Table 1.—Morphometric values (in mm) for *Mesobuthus caucasicus przewalskii* (Tuokexun County, Xia Village, 42°47′N, 88°40′E), *M. caucasicus intermedius* (Almaty Area, Kurty District, 44°53′N, 75°17′E), *M. karshiius* new species (Karshi District, Shache County, 38°24′N, 77°05′E), *M. eupeus mongolicus* (Alxa Youqi, 39°12′N, 101°42′E), *M. eupeus thersites* (Yining County, 44°00′N, 81°31′E), and *M. martensii martensii* (Alxa Zuoqi, 38°39′N, 105°48′E).

	M. caucasicus przewalskii		*M. caucasicus intermedius*		*M. karshiius* new species		*M. eupeus mongolicus*		*M. eupeus thersites*		*M. martensii martensii*	
Sex	♂	♀			♂	♀	♂	♀				
Type	"topotype"	"topotype"	♂	♀	paratype	holotype	"topotype"	"topotype"	♂	♀	♂	♀
Total length	55.03	65.57	59.7	75.69	61.11	67.67	40.33	40.51	37.91	41.91	54.31	56.48
Carapace:												
Length	5.77	7.31	6.69	8.15	6.56	7.89	4.24	4.08	4.23	4.46	5.54	5.69
Anterior width	3.15	4.08	3.69	5.08	3.78	4.67	2.62	2.46	2.46	2.84	3.46	3.23
Posterior width	5.78	7.62	6.7	9.39	6.78	8.44	4.95	4.85	4.77	5.08	5.77	6.85
Metasomal segment I:												
Length	4.08	4.77	5.08	6	4.38	5.56	3.09	3.1	2.81	3.09	4.46	4.08
Width	3.92	4.46	4.46	5.23	4.54	5.22	3.05	2.76	3.14	3.05	3.77	3.85
Metasomal segment II:												
Length	5.01	5.78	5.77	6.77	5.15	6.11	3.43	3.19	3.1	3.33	4.77	4.92
Width	3.77	4.31	4.15	5.01	4.31	5.02	3.01	2.71	3.14	3.04	3.62	3.54
Metasomal segment III:												
Length	5.08	6.01	6.15	6.79	5.46	6.44	3.38	3.33	3.52	3.43	5.15	5.08
Width	3.76	4.31	4.15	4.92	4.23	4.89	3	2.71	3.13	3.05	3.54	3.46
Metasomal segment IV:												
Length	5.39	6.15	6.76	7.46	6.08	7.22	4.19	3.81	4.09	3.81	5.54	5.62
Width	3.69	4.15	4.07	4.77	4.08	4.67	2.99	2.71	3.19	3.04	3.46	3.31
Metasomal segment V:												
Length	6.54	7.32	7.63	8.92	7.15	9.11	4.86	4.52	4.86	4.19	5.92	5.85
Width	3.15	3.54	3.77	4.08	3.54	4.33	2.86	2.71	3.05	2.86	3.23	3.15
Depth	2.77	3.08	3.08	3.46	3.08	3.67	2.24	1.95	2.14	2.14	3.01	2.69
Telson:												
Length	5.85	7.31	7.01	9.08	6.46	7.69	4.52	4.33	4.38	4.52	5.85	6.01
Width	2.31	2.92	2.69	3.23	2.54	3.15	1.91	1.86	2.15	2.14	2.54	2.54
Depth	2.02	2.69	2.46	2.92	2.31	2.85	1.81	1.71	1.8	1.81	2.36	2.31
Aculeus length	3.01	3.69	3.61	4.92	3.23	3.92	2.14	2.01	2.19	2.2	2.62	2.85
Pedipalps:												
Femur length	5.01	5.92	5.85	6.54	5.69	6.38	3.86	3.71	3.33	3.52	5.39	5.23
Femur width	1.46	1.77	1.69	2.08	1.69	2.01	1.19	1.14	1.14	1.2	1.46	1.63
Patella length	5.77	6.85	6.69	7.92	6.38	7.54	4.52	4.33	3.71	4.09	6.02	6.02
Patella width	2.15	2.77	2.62	3.08	2.54	2.54	1.67	1.71	1.67	1.86	2.15	2.39
Chela length	9.85	12.08	11.62	14.23	11.54	12.69	7.85	7.54	6.99	7.46	10.46	10.62
Chela width	2.54	2.92	3	3.23	2.92	3.31	2.15	1.92	2.38	2.19	2.62	2.92
Chela depth	3.08	2.69	3.54	4.02	3.46	3.92	2.46	2.15	2.62	2.39	2.99	2.62
Movable finger length	6.46	8.09	7.31	8.92	7.46	8.23	4.62	4.76	4.27	4.69	6.63	6.92
Pectines:												
Tooth count (L-R)	20-21	17-17	26-26	20-22	25-25	22-21	26-25	20-21	27-27	20-22	25-24	19-20

pectinal teeth number 20–25 in females and 26–30 in males, with 15–19 in females and 19–23 in males in *M. c. przewalskii* (Fig. 15); 2) dentate margins of movable and fixed fingers with 12 and 11 oblique rows of granules respectively, whereas movable and fixed fingers with 11 and 10 oblique rows of granules respectively in *M. c. przewalskii*; 3) aculeus longer than a half of telson length, while aculeus about equal to a half of telson length in *M. c. przewalskii*.

Distribution.—*Mesobuthus caucasicus intermedius* occurs in China (Xinjiang Uygur Autonomous Region), Iran (northwest), Kazakhstan, Kirghizstan, Tajikistan, Turkmenistan, and Uzbekistan.

Discussion.—Although we have conducted fieldwork in Xinjiang and other areas of northwest China over the past four years and have collected a large number of scorpion specimens, we could not find evidence to support a wide

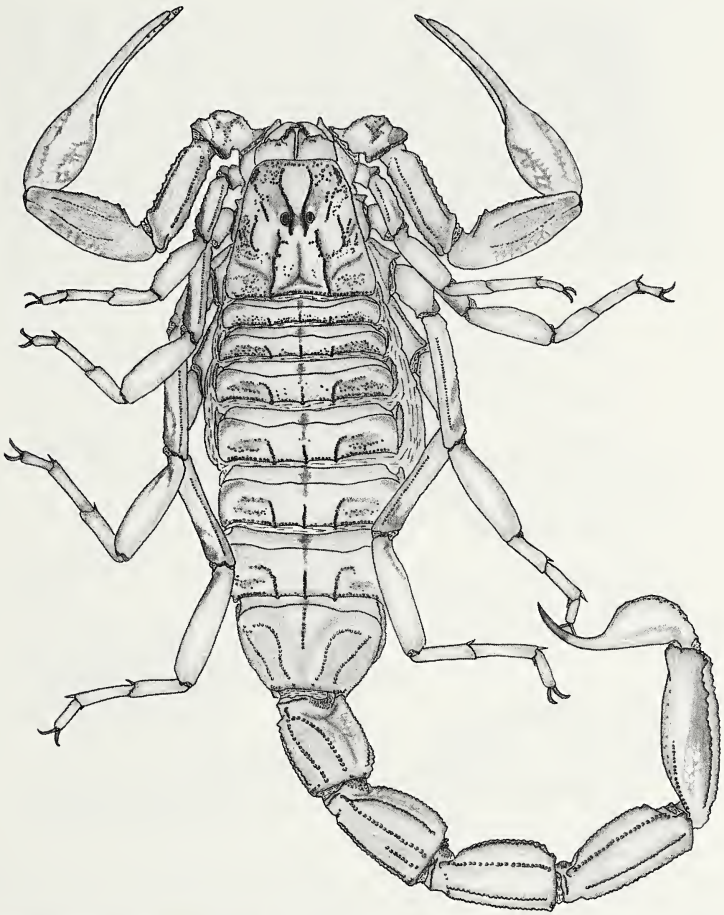

Figure 3.—*Mesobuthus caucasicus intermedius* (Birula 1897), female from Almaty Area, Kurty District (44°53′N, 75°17′E), dorsal view.

distribution of *M. caucasicus intermedius* in China (as in *M. caucasicus przewalskii*).

Mesobuthus karshius new species
(Figs. 5, 6, 10, Table 1)

Material examined.—Holotype ♀ (MHBU), CHINA: *Xinjiang Uygur Autonomous Region*: Karshi District, Shache County, 38°24′N, 77°05′E, 6 August 2006, F. Zhang, H.Q. Ma and S.N. Liu. Paratypes: 27 ♀, 17 ♂ (MHBU), all the same as for holotype; 1 ♀, Karshi District, area near to Karshi City, 39°28′N, 75°58′E, 7 August 2006, F. Zhang, H.Q. Ma and S.N. Liu (MHBU); 8 ♀, 2 ♂, Artush City, area near to Arhu Town, 39°42′N, 76°09′E, 7 June 2009, D. Sun and Y.W. Zhao (MHBU); 3 ♀, 1 ♂, 2 km S of Artush City, near to Songtake Village, 39°41′N, 76°11′E, 8 June 2009, D. Sun and Y.W. Zhao (MHBU).

Etymology.—The specific name refers to Karshi (Kashgar) District, Xinjiang Autonomous Region, China, type locality of the new species.

Diagnosis.—Total length 56–72 mm in females and 46–62 mm in males. General coloration yellow to brownish-yellow; anterior median carinae of carapace with dark brown pigment and other carinae with light brown pigment; metasoma yellow to brownish-yellow, only ventral-median carinae with brown pigment. Prosoma: anterior, central, and

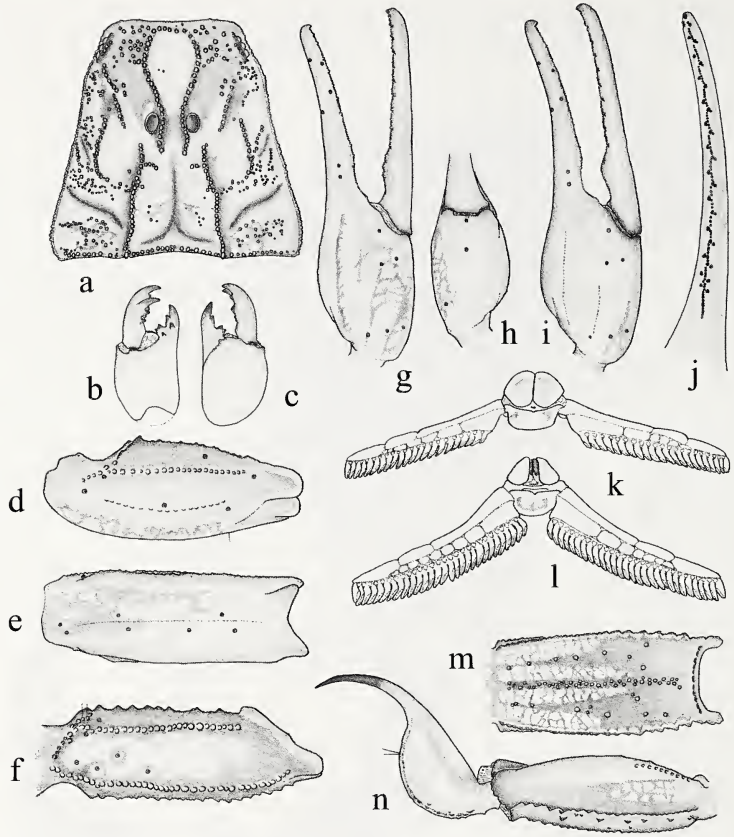

Figure 4.—*Mesobuthus caucasicus intermedius* (Birula 1897), from Almaty Area, Kurty District (44°53′N, 75°17′E): a–h, j, k, m, n: female; i, l: male. a. Carapace, dorsal aspect; b, c. Chelicera (b, ventral; c, dorsal); d, e. Patella (d, dorsal; e, external); f. Femur, dorsal aspect; g–i. Chela (g, i, dorso-external; h, ventral); j. Disposition of granulations on the dentate margins of the pedipalp chela movable finger; k, l: Genital operculum and pectines, ventral aspect; m. Metasomal segment V, ventral aspect; n. Metasomal segment V and telson, lateral aspect.

posterior median carinae granular and granules relatively minor; central median carinae directly connected with posterior median carinae and lateral median carinae by a row of sparse granules. Mesosoma: Tergite: segments I–VI tricarinate; the intercarinal surfaces relatively smooth, except for the posterior margins with fine granules; exterior surfaces with dense granules. Pectines: moderately long; pectinal teeth 19–23 in females and 23–28 in males. Metasoma: Segments I–V with 10-8-8-8-5 complete carinae, median lateral carinae complete on segment I, only with sparse granules and covered 1/2–2/3 length of segment on II, almost obsolete and remaining several granules at distal end on III and absolutely obsolete on IV; ventrolateral carinae on segment V markedly serrate, stronger posteriorly, and posterior lobed granules not uniform; aculeus slightly more than a half of telson length. Dentate margins of movable and fixed fingers with 12 and 11 oblique rows of granules respectively; outer accessory denticles uniform from base to tip (not becoming smaller), and nearly same as inner accessory denticles on the tip in size. Legs: Tarsus with two short longitudinal rows of setae positioned ventrally.

This subspecies is undoubtedly allied with *M. caucasicus*, especially in these characters: the shape of carinae on carapace, the shape of chela and metasoma, and the characters about carinae on metasoma. It can, however, be distinguished by three features:

1) Characters distinguished from *M. c. przewalskii*: a) the carinae and granules on carapace moderately strong, while the carinae and granules on carapace markedly strong in

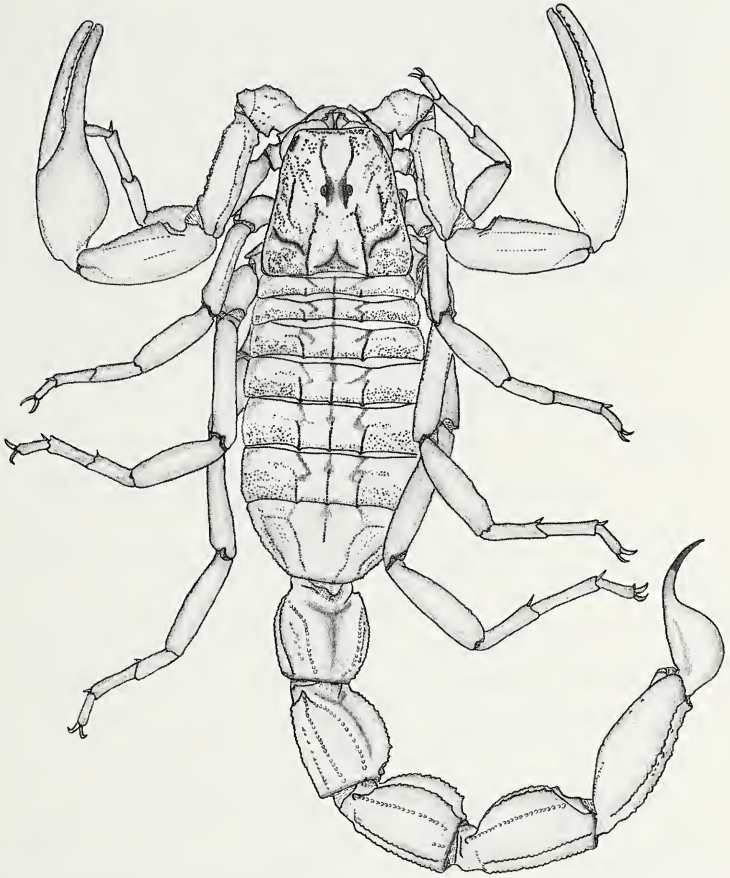

Figure 5.—*Mesobuthus karshius* new species, female holotype from Karshi District, Shache County (38°24′N, 77°05′E), dorsal view.

M. c. przewalskii; b) dentate margin of movable finger of chela with 12 oblique rows of granules, but with 11 oblique rows in *M. c. przewalskii*; c) pectinal teeth 19–23 in females and 23–28 in males, but 15–19 in females and 19–23 in males in *M. c. przewalskii* (Fig. 15); d) the new species without irregular net-like dark pigmentation on chela, dorsal surfaces of segments I–V on metasoma and ventral surface of segment V, while *M. c. przewalskii* with these pigmentation; e) tarsus of legs with two short longitudinal rows of setae, *M. c. przewalskii* with two long longitudinal rows of setae.

2) Characters distinguished from *M. c. intermedius*: a) pectinal teeth 19–23 in females and 23–28 in males, but 20–25 in female and 26–30 in male *M. c. intermedius* (Fig. 15); b) new species without irregular net-like dark pigmentation on chela, dorsal surfaces of segments I–V on metasoma and ventral surface of segment V, while *M. c. intermedius* with this pigmentation; c) chela of new species with outer accessory denticles uniform from base to tip (not becoming smaller) and nearly same as inner accessory denticles on the tip in size, while *M. c. intermedius* with outer accessory denticles becoming markedly smaller from base to tip, and obviously smaller than inner accessory denticles on the tip; d) tarsus of legs with two short longitudinal rows of setae, *M. c. intermedius* with two long longitudinal rows of setae.

3) Characters distinguished from *M. c. parthorum*: a) pectinal teeth 19–23 in females and 23–28 in males, 22–24 in females and 29–34 in males in *M. c. parthorum*; b) general coloration of metasoma is yellow to brownish-yellow, whereas it is markedly dark brown in *M. c. parthorum* (Vachon 1958).

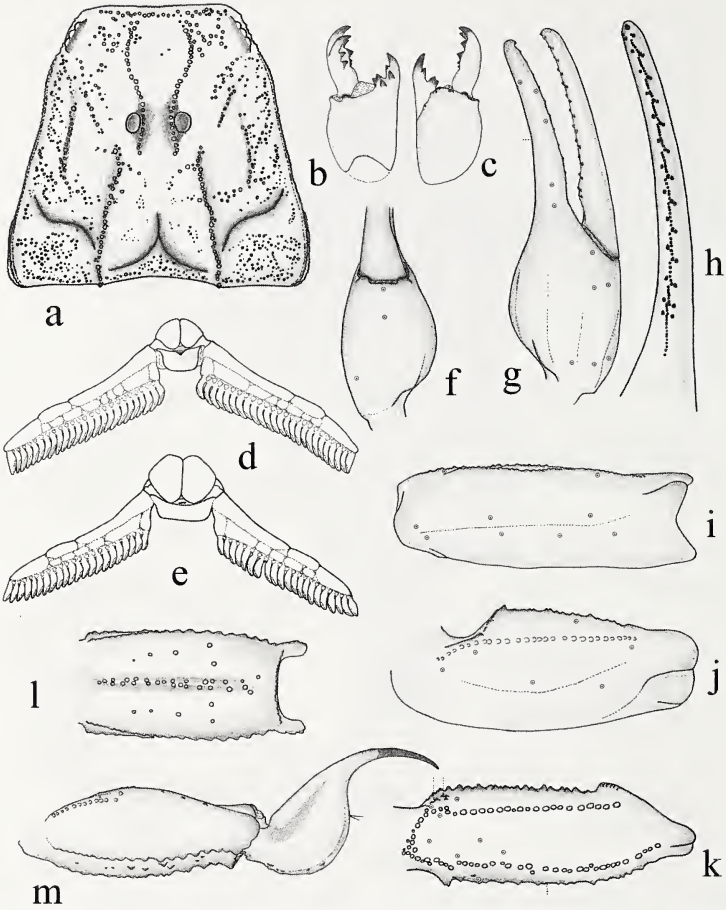

Figure 6.—*Mesobuthus karshius* new species, from Karshi District, Shache County (38°24′N, 77°05′E): a–c, e–m: female holotype; d: male paratype. a. Carapace, dorsal aspect; b, c. Chelicera (b, ventral; c, dorsal); d, e. Genital operculum and pectines, ventral aspect; f, g. Chela (f, ventral; g, dorso-external); h. Disposition of granulations on the dentate margins of the pedipalp chela movable finger; i, j. Patella (i, external; j, dorsal); k. Femur, dorsal aspect; l. Metasomal segment V, ventral aspect; m. Metasomal segment V and telson, lateral aspect.

Description.—Based on female holotype. Species of moderate to large size, with respect to the genus. Total length 56–72 mm in females and 46–62 mm in males.

Coloration: basically yellow to brownish-yellow. Prosoma: carapace brownish-yellow, middle and lateral eyes surrounded by black pigment; anterior median carinae with dark brown pigment and other carinae with light brown pigment. Mesosoma: brownish-yellow; middle and lateral carinae with brown pigment on segments I–VI and without pigment on segment VII. Metasoma: yellow to brownish-yellow, only ventral-median carinae with brown pigment; vesicle light brownish-yellow and aculeus dark reddish on its extremity. Venter brownish-yellow, except for the pectines, which are pale yellow. Chelicerae: light brownish-yellow without pigmentation; teeth dark reddish to brownish. Pedipalps: brownish-yellow without pigmentation; granules on dentate margins of the fingers blackish-brown. Legs: brownish-yellow without pigmentation.

Morphology: Prosoma: anterior margin with a very weak median concavity; carinae moderately strong, only anterior lateral carinae weak; anterior, central and posterior median carinae granular, and granules relatively minor; central median carinae directly connected with posterior median carinae and lateral median carinae by a row of sparse granules;

posterior median carinae terminating distally in a small spinoid process that extends slightly beyond the posterior margin of the carapace; surfaces between median carinae almost smooth, but the external surfaces with comparatively dense small granules; the surfaces between anterior median carinae and lateral eyes coarsely granular; furrows moderate. Median ocular tubercle slightly anterior to the center of carapace; median eyes separated by almost 2.0 ocular diameters; three pairs of lateral eyes.Mesosoma: Tergite: segments I–VI tricarinate; median and lateral carinae on I–VI moderate, granular; each carina on I–VI terminating distally in a small spinoid process, which extends beyond the posterior margin of tergite, except the median carina on I and II; intercarinal surfaces relatively smooth, except for posterior margins with fine granules; exterior surfaces with dense granules; VII pentacarinate; two pairs of lateral carinae moderate to strong; median carinae present on proximal half, moderate; intercarinal surfaces with sparse granules. Sternites: segments III–VII smooth; lateral margins moderately serrate; VII with four moderately marked carinae, granular, and the intercarinal surfaces smooth. Pectines: moderately long; pectinal teeth 19–23 in females and 23–28 in males. Metasoma: Segments I with 10 complete carinae, segments II–IV with 8 complete carinae; all carinae moderately strong, granular, except the dorsal carinae, serrate and stronger posteriorly; median lateral carinae complete on segment I, only with sparse granules and covered 1/2–2/3 length of segment on II, almost obsolete with several remaining granules at distal end on III and absolutely obsolete on IV; intercarinae surfaces on segments I to IV smooth, except the surfaces between dorsal and dorsolateral carinae on segment I, which are weakly to moderately granular. Segment V pentacarinate; ventral carina moderate, granular; ventrolateral carinae markedly serrate, stronger posteriorly, and posterior lobed granules not uniform; dorsolateral carinae weakly developed, little shorter than the length of this segment, obsolete posteriorly; dorsal and lateral surfaces smooth, ventral surface with sparse large granules. Telson smooth dorsally and weakly granular ventrolaterally; aculeus long, slightly more than a half of telson length. Chelicerae: Dentition as defined by Vachon (1963) for the family Buthidae. Pedipalps: Trichobothrial pattern: Orthobothriotaxic A-β (Vachon 1974, 1975). Femur pentacarinate, moderately to strongly granular; ventrointernal carina with spinoid granules. Patella with seven carinae, weakly to moderately granular. Intercarinal surfaces on both segments smooth. Chela smooth without carinae; dentate margins of movable and fixed fingers with 12 and 11 oblique rows of granules respectively; outer accessory denticles uniform from base to tip (not becoming smaller), and nearly same as inner accessory denticles on the tip in size. Legs: Tarsus with two short longitudinal rows of setae positioned ventrally; tibial spurs present on legs III and IV, moderately marked; pedal spurs present and moderately developed on all legs.

Distribution.—*Mesobuthus karshius* occurs in China (Xinjiang Uygur Autonomous Region).

Variation.—The posterior lobed granules on ventrolateral carinae of metasoma segment V in some elderly individuals are relatively smooth, which may result from abrasion after their last ecdyses. This character is not found in juveniles or most adult individuals. Also, there is nothing markedly sexually dimorphic in this variation. Several individuals with light brown to brownish-yellow pigmentation on the ventral surfaces of metasoma segment V, and most individuals without. Sternite segment VII with four moderately granular lateral carinae in adult female individuals, males with four very slightly granular or absolutely no-granular lateral carinae.

Ecology.—The new species is abundant in habitats such as houses built with blocks of soil or stone, in which cement is not used. They were commonly collected in clefts of walls, but also under blocks of soil or stones. In natural environments, most specimens were collected under large blocks of soil or stones; however, a few specimens were found under small blocks of soil or stones.

Mesobuthus eupeus mongolicus (Birula 1911)
(Figs. 7, 8, 10, Table 1)

Buthus eupeus mongolicus Birula 1911:195; Birula 1917:42; Birula 1925:96; Birula 1927:202; Takashima 1945:77.

Buthus (*Buthus*) *eupeus mongolicus* Birula: Birula 1917:239.

Mesobuthus eupeus mongolicus (Birula): Vachon 1958:155, fig. 37; Stahnke 1967:61–68, figs. 1–5; Pérez 1974:27; Farzanpay 1986:334; Fet 1994:527; Kovařík 1997:180; Kovařík 1998:114; Fet & Lowe 2000:174; Gantenbein et al. 2003:413, table 1; Qi et al. 2004:138, 142; Zhu et al. 2004:112; Shi & Zhang 2005:474; Parmakelis et al. 2006:2886, 2889, fig. 2, table 1; Shi et al. 2007:216, 218; Sun & Zhu 2010:2.

Type specimens.—Type material not examined.

Material examined.—CHINA: *Inner Mongolia Autonomous Region*: Wuhai City, 10 km E of downtown area, Zhuozi hill, 39°40′N, 106°56′E, 19 July 2007, Z.Y. Di, Y.N. Fu and M.C. Xie, 12 ♀, 9 ♂, 2 juveniles (MHBU); Urad Zhongqi, Wujiahe Town, north hill (part of Yin Mountain), 41°16′N, 108°13′E, 16 July 2008, D. Sun and C.L. Zhang, 16 ♀, 17 ♂ and 6 juveniles (MHBU); Wuhai City, south park of locomotive depot (in sandy tracts), 39°38′N, 106°48′E, 22–23 July 2008, D. Sun and C.L. Zhang, 20 ♀, 22 ♂, 10 juveniles (MHBU); Alxa Zuoqi, Wenduermaodao District, 40°54′N, 104°20′E, 26 July 2008, M.S. Zhu and D. Sun, 2 ♀ (MHBU); Urad Houqi, 15 km S of Chaogewenduer Town, south hill (part of Lang Mountain), 41°19′N, 107°04′E, 21 July 2008, D. Sun and C.L. Zhang, 3 ♀, 2 juveniles (MHBU); Bayan nur League, Dengkou County, Bayan ula (township level village), 40°22′N, 106°57′E, 25 July 2008, M.S. Zhu and D. Sun, 1 ♀, 1 ♂, 1 juvenile (MHBU). *Gansu Province*: Zhangye City, 38°56′N, 100°23′E, August 2005, collector unknown, 2 ♀ (MHBU); Jiuquan City, Suzhou District, 5 km S of Qingshui Town, Qilin Township, 39°18′N, 99°01′E, 13 August 2008, M.S. Zhu and D. Sun, 10 ♀, 9 ♂, 4 juveniles (MHBU); Jiuquan City, Jinta County, Yuanyangchi Scenic Spot, 39°50′N, 98°52′E, 28 July 2008, M.S. Zhu and D. Sun, 1 ♀, 3 ♂ and 1 juvenile (MHBU). *Ningxia Hui Autonomous Region*: Helan Mountain National Nature Reserve, Liutiao Clough, exact location unknown, 29 July 2008, X.P. Wang, 3 ♀, 4 ♂ (MHBU). *Xinjiang Uygur Autonomous Region*: Tuoli County, area 10 km SE of Tiechanggou Town, 46°06′N, 84°33′E, 9 August 2008, M.S. Zhu and D. Sun, 4 ♀, 2 ♂ (MHBU); Manas County, South Hill Ranch, 43°55′N, 85°51′E, 10 August 2008, M.S. Zhu and D.

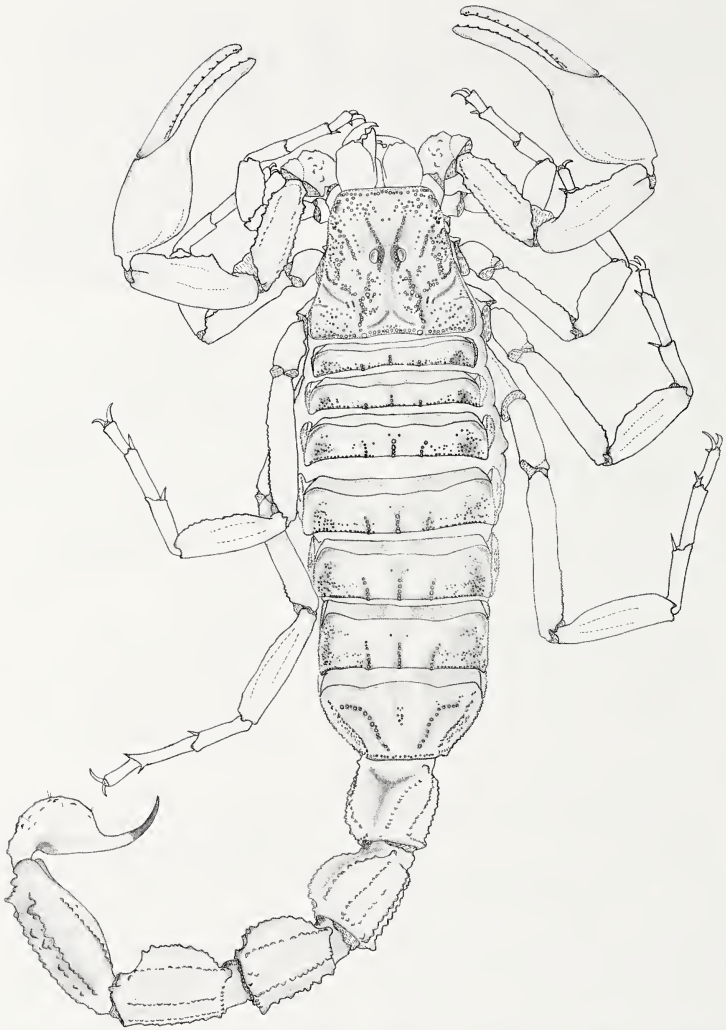

Figure 7.—*Mesobuthus eupeus mongolicus* (Birula 1911), female "topotype" from Alxa Youqi (39°12′N, 101°42′E), dorsal view.

Sun, 4 ♀, 4 ♂, and 6 juveniles (MHBU); Bole City, 10km S of Bole downtown area, desolated sands, 44°47′N, 82°02′E, 4 August 2007, D. Sun and L. Zhang, 3 ♀, 1 ♂, and 7 juveniles (MHBU); Karamay City, 2–3 km N of downtown area, 45°38′N, 84°51′E, 30 July 2007, D. Sun and L. Zhang, 1 ♀, 1 juvenile (MHBU); Tuoli County, "Dongwozi" Ranch, exact location unknown, 30 July 2007, D. Sun and L. Zhang, 3 ♀, 2 ♂ (MHBU); Fuhai County, Wucaiwan, desolated sands, 47°50′N, 86°40′E, 20 July 2007, D. Sun and L. Zhang, 2 ♂ (MHBU); Tuoli County, exact location unknown, 20 August 2006, F. Zhang, H.Q. Ma and S.N. Liu, 1 ♂ (MHBU); Urumqi County, Liuhuang Clough, 43°44′N, 87°13′E, 1 September 2006, F. Zhang, H.Q. Ma and S.N. Liu, 13 ♀, 9 ♂ (MHBU); Alataw Pass, near the frontier inspection station of China, 45°02′N, 82°34′E, 5 August 2007, D. Sun and L. Zhang, 4 ♀, 1 ♂, 16 juveniles (MHBU); Wenquan County, exact location unknown, 16 August 2006, F. Zhang, H.Q. Ma and S.N. Liu, 1 ♀, 1 ♂ (MHBU); Qinghe County, 46°40′N, 90°20′E, 11 June

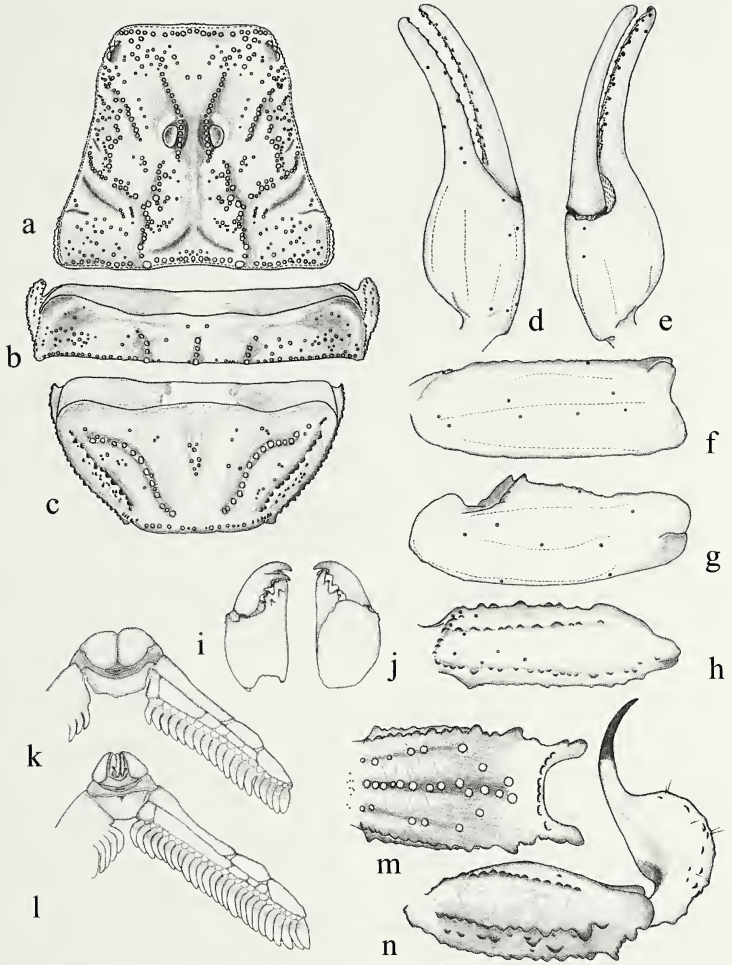

Figure 8.—*Mesobuthus eupeus mongolicus* (Birula 1911), from Alxa Youqi (39°12′N, 101°42′E): a–k, m, n: female "topotype"; l: male "topotype". a. Carapace, dorsal aspect; b. Segment III of tergite, dorsal aspect; c. Segment VII of tergite, dorsal aspect; d, e. Chela (d, dorso-external; e, ventral); f, g. Patella (f, external; g, dorsal); h. Femur, dorsal aspect; i, j. Chelicera (i, ventral; j, dorsal); k, l. Genital operculum and pectines, ventral aspect; m. Metasomal segment V, ventral aspect; n. Metasomal segment V and telson, lateral aspect.

2006, Y.B. Ba, 1 ♂ (MHBU); Fuyun County, 46°58′N, 89°31′E, 6 June 2006, Y.B. Ba, 1 ♀, 1 ♂ (MHBU); Alataw Pass, 45°09′N, 82°36′E, 15 August 2006, F. Zhang, H.Q. Ma and S.N. Liu, 1 ♂ (MHBU). Other material examined, see Zhang & Zhu (2009).

Diagnosis.—Total length 40–55 mm in females and 35–45 mm in males. General coloration light yellow to pale brownish-yellow; anterior median, central median and posterior median carinae of carapace with dark brown pigment; tergite I–VI segments with 3 or 5 longitudinal dark brown strips (one of the most conspicuous characters). Prosoma: anterior margin with a very weak median projection or approximately straight, all carinae weak to moderately strong, posterior median carinae terminating distally in a small spinoid process not extending beyond the posterior margin of the carapace. Mesosoma: Tergite segments I–VI tricarinate; intercarinal surfaces with sparse small granules; carinae terminating distally in small spinoid process that does not

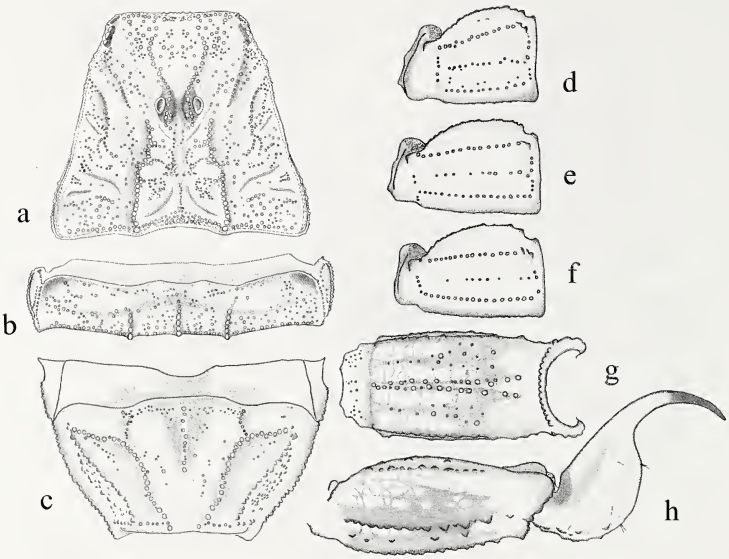

Figure 9.—*Mesobuthus martensii martensii* (Karsch 1879), female from Alxa Zuoqi (38°39′N, 105°48′E). a. Carapace, dorsal aspect; b. Segment III of tergite, dorsal aspect; c. Segment VII of tergite, dorsal aspect; d–f. Metasomal segments, lateral aspect (d, segment I; e, segment II; f, segment III); g. Metasomal segment V, ventral aspect; h. Metasomal segment V and telson, lateral aspect.

extend beyond posterior margin of carapace. Pectines: moderately long; pectinal teeth 19–22 in females and 24–28 in males. Metasoma: Segments I–V with 10-8-8-8-5 complete carinae, median lateral carinae complete on segment I, only with sparse granules and covered half length of segment or little more on II, almost obsolete with several remaining granules at distal end on III and absolutely obsolete on IV; ventral carinae on segment II and III with markedly serrate granules, becoming larger posteriorly; ventrolateral carinae on segment V strong, serrate, becoming strongly marked posteriorly with 1–3 (mostly 2) markedly large and extroversive lobed granules; aculeus equal to half of telson length. Dentate margins of movable fingers with 10–11 (mostly 10) oblique rows of granules. Legs: Tarsus with two short, strong longitudinal rows of setae positioned ventrally.

Distribution.—*Mesobuthus eupeus mongolicus* occurs in China (Inner Mongolia Autonomous Region, Gansu Province, Ningxia Hui Autonomous Region, Xinjiang Uygur Autonomous Region), Mongolia.

Variation.—The marked variant character among different geographical populations is the dark or light brown pigmentation on the ventral surfaces of metasoma segments I–IV. Individuals from the type locality ("Alasham Province" in about 1907, and "Alxa Zuoqi" today) and nearby areas (Inner Mongolia Autonomous Region, Gansu Province, Ningxia Hui Autonomous Region) are without the brown pigmentation, whereas some of the specimens collected from Xinjiang Uygur Autonomous Region have dark or light brown pigmentation, or lack pigmentation.

Ecology.—The distribution of this subspecies is from southern Mongolia, Inner Mongolia Autonomous Region, Ningxia Hui Autonomous Region, northern and western of Gansu Province to eastern and northern Xinjiang Uygur Autonomous Region. This area is an arid or semi-arid continental climatic region: hot and arid in summer, cold and dry in winter, and quite windy and dusty in spring. According to this rhythm, the main active period of individuals is from early April to early October. Typically, this subspecies inhabits a terrene hillside with crushed rocks and low herbaceous plants, and it often hides in a flat hole under rocks during the day.

Mesobuthus eupeus thersites (C.L. Koch 1839)
(Fig. 10, Table 1)

Androctonus thersites C.L. Koch 1839:51, plate CXCIII, fig. 466 (synonymized by Birula 1896:238); Kraepelin 1891:204.

Buthus eupeus thersites (C.L. Koch): Kraepelin 1899:24; Birula 1900:359; Birula 1904:20; Birula 1905:122, fig. 3; Birula 1906:45, plate V, fig. 1; Roewer 1943:206.

Buthus eupeus volgensis Birula 1925:96 (synonymized by Birula 1928:338).

Mesobuthus eupeus thersites (C.L. Koch): Vachon 1958:155, fig. 37; Pérez 1974:26; Fet 1980:224; Farzanpay 1986:334; Farzanpay 1988:38; Fet 1989:91; Fet 1994:527; Kovařík 1997:49; Kovařík 1998:114; Fet & Lowe 2000:175; Gantenbein et al. 2003:413, 417, table 1; Qi et al. 2004:138, 142; Zhu et al. 2004:112–113; Shi & Zhang 2005:474–475; Parmakelis et al. 2006:2886, 2889, fig. 2, table 1; Shi et al.

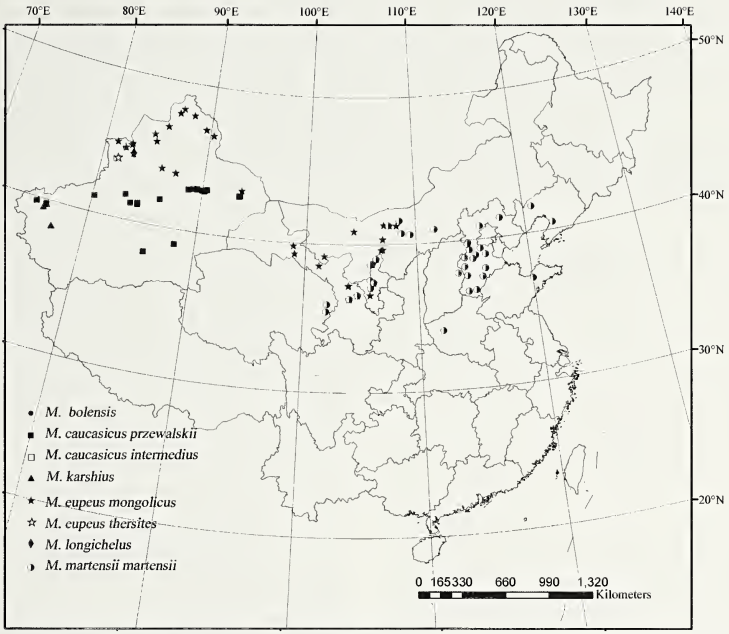

Figure 10.—Map of China illustrating the recorded distributional ranges of the genus *Mesobuthus* in China.

2007:216, 219; Kamenz & Prendini 2008:8, 41, plate 32; Sun & Zhu 2010:2.

Mesobuthus eupeus volgensis (Birula): Orlov & Vaslilyev 1983:62.

Type specimens.—Type material not examined.

Material examined.—CHINA: *Xinjiang Uygur Autonomous Region*: Yining County, north of county cement works, hillsides, 44°00′N, 81°31′E, 18 May 2009, D. Sun and Y.W. Zhao, 10 ♀, 12 ♂ (MHBU). UZBEKISTAN: *Bukhara Area*: Gizhduvan District, SW foothills of Karatau Mountain Range, 14.5 km N of Kanimekh, 5 June 2003, L. Prendini and A.V. Gromov, 3 ♀, 4 ♂ (AMNH). KAZAKHSTAN: *Almaty area*: Kurty District, Taukum Desert, 25.5 km SE of Topar, 9 May 2003, L. Prendini and A.V. Gromov, 2 ♀, 3 ♂ (AMNH); South Kazakhstan area: Suzak District, SW slope of Togyzkentau Mountain Range, 30 km SSW of Sholakespe village, 24 June 2003, L. Prendini and A.V. Gromov, 3 ♀, 1 male (AMNH); Otrar District, 4.5 km SSE of Utrabat (32 km SSE of Turkestan), Sargatazhol boundary, 21 June 2003, L. Prendini and A.V. Gromov, 2 ♀, 3 ♂ (AMNH).

Diagnosis.—This subspecies is associated with *M. eupeus mongolicus*, especially in the following characters: a) the shape and development of carinae on carapace and tergites; b) the numbers of pectinal teeth in males and females; c) the shape and development of carinae on metasoma segments I–V, especially the ventral carinae on segments II–III and ventrolateral carinae on segment V.

The subspecies can be distinguished by the following three features:

1) Anterior margin of carapace in *M. e. thersites* with very weak median concavity; while that of *M. e. mongolicus* either has a very weak median projection or is approximately straight.
2) Chela of *M. e. thersites* more robust; *M. e. mongolicus* with relatively less robust chela (Table 1).
3) Dorsal carinae on metasoma segments I–IV of *M. e. thersites* relatively weak, approximately obsolete anteriorly, moderately granular posteriorly; in contrast, dorsal carinae on metasoma segments I–IV of *M. e. mongolicus* much developed, moderately granular anteriorly, and with marked granules posteriorly.

Distribution.—*Mesobuthus eupeus thersites* occurs in China (Xinjiang Uygur Autonomous Region), Kazakhstan, Uzbekistan, Tajikistan and Kyrgyzstan.

Discussion.—According to the analysis of some species and subspecies of *Mesobuthus* based on molecular data by Gantenbein et al. (2003), the relationship between *M. e. thersites* and *M. e. mongolicus* is not very clear. After inspecting a significant number of specimens of these two subspecies from extensiveness regions, we discovered diagnostic characteristics (above) that were consistent among different geographical populations.

Mesobuthus longichelus Sun & Zhu 2010
(Fig. 10)

Mesobuthus longichelus Sun & Zhu 2010:5–10, figs. 1, 4–10, 17–21; Sun et al. 2010:36, 38–40, figs. 4, 12, 13, 19, 20, 23, 24, table 1.

Material examined.—See Sun & Zhu (2010).

Diagnosis.—See Sun & Zhu (2010).

Distribution.—*Mesobuthus longichelus* occurs in China (Xinjiang Uygur Autonomous Region).

Ecology.—See Sun & Zhu (2010).

Mesobuthus martensii martensii (Karsch 1879)
(Figs. 9, 10, Table 1)

Buthus martensii Karsch 1879:112; Kishida 1939:51–67, plate I–IV.

Buthus confucius Simon 1880:124–125 (synonymized by Karsch 1881:219).

Buthus conficius [sic] Simon: Pocock 1889a:336–337, plate X–V, fig. 2a; Pocock 1889b:116; Birula 1898:133–134; Birula 1927:205–209; Kästner 1941:231.

Buthus martensi Karsch: Kraepelin 1899:25–26; Wu 1936:115–117, fig. 1; Takashima 1944:51–53; Takashima 1945:75; Vachon 1948:61, fig. 4; Isshiki & Yonezawa 1960:117–123; Song *et al.* 1982:22–25, figs. 1–7; Song 1998:508, fig. 30:1.

Buthus nigrocinctus (nec *Androctonus nigrocinctus* (Ehrenberg 1828): Thorell 1893:360–361.

Mesobuthus martensi (Karsch): Vachon 1950:153; Vachon 1952:325; Pérez 1974:26; Kovařík 1992:183.

Mesobuthus martensii (Karsch): Kovařík 1998:115; Shi & Zhang 2005:474; Shi et al. 2007:216–223, figs. 1–3, table 1; Zhang & Zhu 2009:1–17, figs. 1–18, tables 1–8; Sun & Zhu 2010:10.

Mesobuthus martensii martensii (Karsch): Fet & Lowe 2000:178; Qi et al. 2004:137–143, figs. 1–19, table 1; Zhu et al. 2004:113.

Type specimens.—Type material not examined.

Material examined.—CHINA: *Gansu Province*: Jingyuan County, Mitan Township, 36°35′N, 104°40′E, 5 August 2007, Z.Y. Di, Y.N. Fu and M.C. Xie, 1 ♀, 2 ♂ (MHBU); Gaolan County, Dongwan Village, 36°20′N, 103°57′E, 4 August 2007, Z.Y. Di, Y.N. Fu and M.C. Xie, 1 ♀, 1 ♂ (MHBU). *Ningxia Hui Autonomous Region*: Yinchuan City, Helan Mountain National Nature Reserve, Suyukou forest park, 38°42′N, 105°57′E, 14–17 August 2008, X.P. Wang and G.J. Yang, 10 ♀, 9 ♂, 1 juvenile (MHBU); Helan Mountain National Nature Reserve, Liutiao Clough, exact location unknown, 29 July 2008, X.P. Wang, 9 ♀, 10 ♂ (MHBU). *Inner Mongolia Autonomous Region*: Alxa Zuoqi, Nansi, 38°39′N, 105°48′E, 21 July 2007, Z.Y. Di, Y.N. Fu and M.C. Xie, 25 ♀, 21 ♂, 8 juveniles (MHBU); Urad Zhongqi, Hailiutu Town, north hill (part of Yin Mountain), 41°36′N, 108°30′E, 15 July 2008, D. Sun and C.L. Zhang, 1 male (MHBU); Baotou City, Jiuyuan District, Agerutai Sumu, Meiligeng Gacha, 40°38′N, 109°27′E, Tongla and J.J. Wang, 15 August 2006, 3 ♀, 4 ♂, 3 juveniles (MHBU); Urad Zhongqi, Shilanji Township, north hill (part of Yin Mountain), 41°17′N, 107°29′E, 18 July 2008, D. Sun and C.L. Zhang, 1 ♀, 3 juveniles (MHBU). *Shandong Province*: Pingdu County, Daze Mountain, 36°59′N, 120°01′E, 5 May 2007, F.Y. Wang, 1 ♂ and 4 juveniles (MHBU). *Hebei Province*: Quzhou County, Anzhai Town, Guzhuang Village, 36°38′N, 115°01′E, August 2004, X.Y. Gu, 19 ♀, 13 ♂ (MHBU); Chicheng County, 40°54′N, 115°50′E, 2 October 2002, Z.S. Zhang, 2 ♀, 3 juveniles (MHBU); Longhua County, 41°18′N, 117°45′E, 14 June 2004, W.G. Lian, 5 ♀, 2 ♂ (MHBU); Handan City, date and collector unknown, 4 ♀, 1 ♂ (MHBU); Laishui County, 39°24′N, 115°42′E, 28 June 2004, J. Song, 20 ♀, 7 ♂ (MHBU); Xiong County, 38°59′N, 116°07′E, 20 July 2004, C.Y. Fan, 3 ♀, 2 ♂ (MHBU); Zhou County, Xiaowutai Mountain National Nature Reserve, 39°50′N, 114°37′E, 10 July 2004, F. Zhang, 21 ♀, 5 ♂ (MHBU); Laiyuan County, 39°21′N, 114°41′E, date and collector unknown, 2 ♀ (MHBU). Liaoning Province: Yingkou City, Dashiqiao County, Laodong Village, 40°30′N, 122°30′E, 14 July 2009, D. Sun, 8♀, 12 ♂ (MHBU). *Shanxi Province*: Yangquan City, 37°51′N, 113°33′E, 3 May 2004, S.J. Zhao, 1 ♀, 2 juveniles (MHBU). *Henan Province*: Song County, Dazhang Township, Baligou, 34°04′N, 111°56′E, 12 July 2004, M.S. Zhu, 3 ♀, 2 ♂ (MHBU). Other material examined, see Zhang & Zhu (2009).

Diagnosis.—See Qi et al. (2004).

Distribution.—*Mesobuthus martensii martensii* occurs in China (north, northeast, northwest), Mongolia, the Korean Peninsula and Japan. In China, *M. martensii martensii* appears to be restricted to south of latitude 43° N and the north side of the Yangtze River, bordered by Helan Mountain, the Tengger and Mo Us Desert in the west and limited by the sea in the east (Shi et al. 2007).

Ecology.—This species was found mainly in habitats composed of temperate and subtropical areas, often under rocks on sunny hillsides with many herbs and shrubs, but without leafy trees in natural environments. Few individuals were found on shaded hillsides of collecting locations, probably because of the diseases and mycotic infections caused by excessive humidity there (Song 1982). The burrow of *M. martensii martensii* often has an underground passageway, generally 30–50 cm below ground level, where they can move to the deepest points when preparing for hibernation in late autumn.

Mesobutlus martensii hainanensis (Birula 1904)

Buthus confucius hainanensis Birula 1904:27.

Mesobuthus martensii hainanensis (Birula): Fet & Lowe 2000:178; Qi et al. 2004:138, 142; Zhu et al. 2004:113; Zhang & Zhu 2009:1; Sun & Zhu 2010:2.

Material examined.—No material examined; type material preserved in Zoological Institute, Russian Academy of Sciences, St. Petersburg, Russia (lost).

Discussion.—The type material was collected on Hainan Island (Hainan Province) by O. Herz in 1895. In the original diagnosis by Birula (1904), only general coloration was used and only a single specimen of unknown sex was investigated. This subspecies remains, however, of dubious validity, mainly because it was never found again on Hainan Island or from adjacent areas, but also because no species of *Mesobuthus* has ever been found inhabiting evergreen rain forests (Hainan Island is covered in rainforests and rubber plantations).

KEY TO CHINESE SPECIES AND SUBSPECIES OF *MESOBUTHUS*

1. Ventrolateral carinae of segment V on metasoma strong, serrate, becoming marked posteriorly and with several markedly large and extroversive lobed granules (Figs. 8m, 8n) 2
   Ventrolateral carinae of segment V on metasoma strong, serrate, becoming gradually stronger posteriorly, and without any markedly large and extroversive lobed granules (Figs. 2l, 2m) 4
2. Ventral carinae of segment II and III on metasoma gradually stronger posteriorly (Fig. 7) 3
   Ventral carinae of segment II and II on metasoma not stronger posteriorly (Sun & Zhu 2010, fig. 1) ... *Mesobuthus longichelus*
3. Anterior margin of carapace with a very weak median concavity; chelae more robust (Table 1) .... *Mesobuthus eupeus thersites*
   Anterior margin of carapace with a very weak median projection or approximately straight (Fig. 8a); chelae relatively less robust (Table 1) ..... *Mesobuthus eupeus mongolicus*
4. Ventral surface of segment V on metasoma without brown pigment (Fig. 6l) 5
   Ventral surface of segment V on metasoma with markedly brown pigment (Figs. 2l, 4m, 9g) 6
5. Surfaces of carapace with relatively dense small granules (Sun et al. 2010, figs. 2, 3); tarsus of legs with two long longitudinal rows of setae positioned ventrally ..... *Mesobuthus bolensis*
   Surfaces of carapace between median carinae almost smooth, but the external surfaces with comparatively dense small granules (Fig. 6a); tarsus of legs with two short longitudinal rows of setae positioned ventrally ..... *Mesobuthus karshius* new species
6. Dorsal surfaces of metasomal segments I–IV and each surface of segment V with irregular net-like dark pigmentation (Fig. 2k) 7
   Only surfaces of segment V on metasoma with irregular net-like dark pigmentation, dorsal surfaces of segments I–IV without net-like pigmentation ..... *Mesobuthus martensii*
7. Pectinal teeth number 20–25 in females and 26–30 in males; dentate margins of movable and fixed fingers with 12 and 11 oblique rows of granules respectively ..... *Mesobuthus caucasicus intermedius*
   Pectinal teeth number 15–19 in females and 19–23 in males; dentate margins of movable and fixed fingers with 11 and 10 oblique rows of granules respectively ..... *Mesobuthus caucasicus przewalskii*

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Egg capsule architecture and siting in a leaf-curling sac spider, *Clubiona riparia* (Araneae: Clubionidae)

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Abstract. Females of the leaf-curling sac spider *Clubiona riparia* build three-sided capsules, in which they enclose both themselves and their eggs. A capsule is usually constructed by bending a single blade of grass or other leaf twice, each time causing a fold that is perpendicular to the long axis of the blade, and joining the edges with silk. When constructed with monocot leaf blades, the resulting capsule is roughly triangular in cross section and 2–4 times as long as it is wide. We sampled occupied capsules from a 0.16–hectare marsh in central Ontario, Canada. Although we found capsules built with the leaves of cattails (*Typha latifolia*), iris (*Iris versicolor*), a grass (*Calamagrostis* sp.), and an unidentified willow shrub (*Salix* sp.), for the current analysis we concentrated on the monocots because of their structural similarity. Capsules built on cattails (2.13 ± 0.14 ml) were more voluminous than those on iris (1.63 ± 0.14 ml), and capsules made of grass blades (0.67 ± 0.08 ml) were the smallest. Nearly 70% of the total variation in capsule volume was associated with differences between the plant species. Only among capsules built on cattails was there a significant positive relationship between pre-oviposition spider mass and capsule volume; it accounted for about 37% of the variability in capsule volume. On willow leaves, spiders always constructed capsules with the lower surface of the leaf to the inside of the capsule; and on cattail blades, spiders always made their bends in a clockwise direction. We discuss the implications of our findings for an understanding of the choices these spiders make just prior to oviposition.

Keywords: Reproductive ecology, parental care, oviposition site choice, clutch mass

Animal architecture has been extensively studied (von Frisch 1974; Collias & Collias 1976; Jones et al. 1997; Hansell 2005; Gould & Gould 2007), with particular attention paid to the structures built by birds (e.g., Hansell 2000), social insects (e.g., Jones & Oldroyd 2007), and web-building spiders (e.g., Kaston 1964; Blackledge & Eliason 2007; Harmer & Herberstein 2009). Among spiders, web building is only one of several architectural modes and at least two of these, burrow excavation and the construction of aerial shelters made with non-silk "decorations" or by leaf curling, involve the use of environmental (as opposed to secreted) materials. Unlike webs, which always serve foraging functions (Eberhard 1990; Foelix 1996) and frequently double as intraspecific communication channels (Witt & Rovner 1982; Foelix 1996), burrows and aerial retreats are usually defensive, serving to protect against predators and parasitoids, excessive thermal load, desiccation, and other threats to the spiders' well being (Morse 1985, 1988; Konigswald et al. 1990; Lubin et al. 1991, 1993; Ward & Lubin 1993).

Aerial shelters or retreats are particularly interesting because, relative to retreats constructed at the soil surface or under rocks or logs, they display the interplay between added exposure to wind, insolation, and visually orienting predators and parasitoids on the one hand, and on the other hand reduced exposure to ground-foraging predators, high soil-surface temperatures, some potential prey items and, possibly, prospective mates (Henschel et al. 1992; Ward & Henschel 1992; Ward & Lubin 1993; Konigswald et al. 1990; Morse 1985, 1988, 2007).

The leaf-curling sac spider, *Clubiona riparia* L. Koch 1866 (Araneae: Clubionidae), is known among arachnologists largely because of the elegant and simple capsule that the female constructs as a shelter for herself and her eggs (Fig. 1: Comstock 1948; Edwards 1958; Dondale & Redner 1982; Paquin & Dupérré 2003). These retreats are constructed by bending a leaf (often of a monocot) twice, thereby forming a chamber that is roughly triangular in cross section, and sealing its seams with silk, with the eggs and female inside (Comstock 1948). The capsule takes time and energy to construct and ultimately bears all of the spider's lifetime reproductive output, assuming the validity of Comstock's assertion that it serves "as a nursery for the spiderlings and a coffin for the parent" (Comstock 1948:581). In that context, the capsule can be viewed as the consummation of a series of choices made by the gravid female — what plant to use as substrate; how high on the plant to build; how large to make the capsule; how tightly to seal its edges with silk — all interconnected and presumably all under the influence of natural selection.

We report here on *C. riparia*'s use of the leaves of three monocots (cattail, *Typha latifolia,* iris, *Iris versicolor*, and a grass, *Calamagrostis* sp.), and to some extent on their use of the leaves of a dicot (an unidentified willow, *Salix* sp.), in constructing enclosed capsules suitable for egg development and protection. Our emphasis here is on capsule volume and its correlates —subsequent papers will cover the energetics of capsule construction and the possibility that the gravid spiders show preferences among the available plant species.

METHODS

Field site and sampling.—The study site was an elongated marsh, 0.16 ha in area, on a small island located at 45°27′33.1″ N, 80°25′52.7″W, about 2.7 km off the northeast shore of

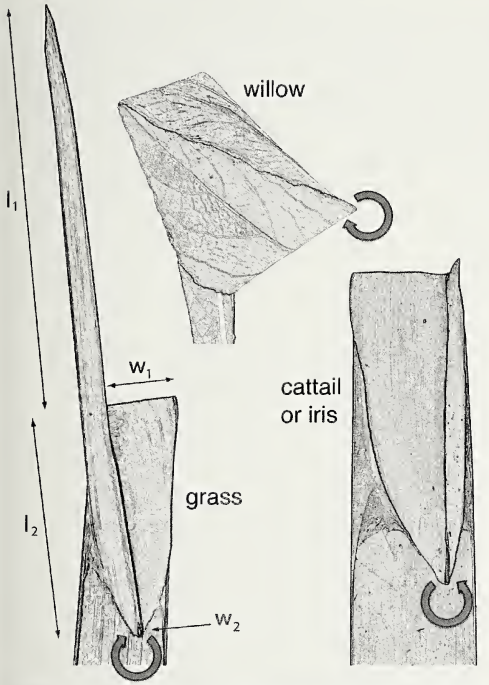


Figure 1.—Capsules of *C. riparia* showing their typical three-sided structure. The circular arrows are included to clarify the convention used to distinguish capsules that are built using clockwise bends (in these examples, grass and willow) from those built using counterclockwise bends (in this example, cattail or iris). The linear dimensions associated with the grass image are those we used to indicate where on a monocot blade the capsule was constructed and to calculate the volume of the capsule (see text).

Georgian Bay, Ontario, Canada. The water of the marsh was confluent with the open waters of Georgian Bay, but sheltered from any wave action. The site was about 10% open, with the remainder covered by vegetation. In terms of plant coverage, the dominant plant was a grass, *Calamagrostis* sp. (monocot, Poaceae). At the north end of the marsh was a stand of cattails, *Typha latifolia* L. (monocot, Typhaceae), covering about 16 m^2, and at various sites in the marsh were clumps of iris, *Iris versicolor* L. (monocot, Iridaceae) and individuals of an unidentified willow shrub, *Salix* sp. (dicot, Salicaceae). Sedges (Cyperaceae) and rushes (Juncaceae), as well as at least one other species of grass (Poaceae), were also present. Each cattail, each iris, and each willow was surrounded by *Calamagrostis* sp., although across much of the area of the marsh, each individual *Calamagrostis* sp. was surrounded only by others of the same species.

Our visual search for the egg capsules of *C. riparia* was careful but not structured. We found capsules on each of the dominant plant species (above), but none on the other grasses, sedges, or rushes. We marked each capsule site with flagging tape and did not return to it until we had searched the entire marsh. Then, as we collected each capsule, we recorded the plant species and the capsule's height above the water surface.

Measurements and analyses.—In the laboratory, we photographed each capsule and used a caliper to measure its linear dimensions to the nearest 0.1 mm. For capsules constructed on monocot blades, these were: leaf tip to capsule, width of the leaf at the first bend, width at the second bend, and capsule length (Fig. 1). We also noted whether the capsule was constructed using a pair of clockwise bends or a pair of counterclockwise bends (Fig. 1) and whether the bends were made in such a way that the top surface of the leaf formed the inside or, conversely, that it formed the external surface of the chamber (we did not score this attribute for cattail or iris blades because we could not differentiate the two surfaces). Finally, we opened each capsule and weighed the spider and the clutch of eggs, each to the nearest mg. In a few cases, the spider had not yet laid its eggs, so for these we recorded gravid female mass as the combined mass of egg clutch and spider (in our analyses, we considered gravid female mass as being equivalent to the sum of egg clutch mass and spider mass when the latter were measured separately).

In calculating the volume of each of the capsules constructed with monocot blades, we first applied Heron's Formula for the area of a triangle (Dunham 1990), assuming the cross-section of the capsule to be an equilateral triangle with side lengths equal to the average of the two widths measured above. We then multiplied this area by the capsule length to get an estimate of the volume. This was an estimate because a) the monocot blades are somewhat tapered, more toward their tips than further down the leaf; b) near the ends of the capsule two sides of the structure converge, giving the cross-section a far less equilateral shape; and c) away from the ends of a capsule, the sides bulge slightly, giving the capsule's cross-section a shape similar to a Reuleaux triangle (i.e., slightly convex on each side; Weisstein 2009).

The most regular of the capsules constructed of willow leaves are approximately tetrahedral in shape (Fig. 1), but many were quite irregular, sometimes more conical or even cylindrical. To measure their volumes, we preserved them in 95% alcohol, then dried them and lightly coated them with silicone (Ace® Silicone Lubricant) to render their surfaces hydrophobic. Finally, we submerged each in a graduated cylinder containing distilled water and measured its volume directly. These volumes are reported below, but in our subsequent analyses we concentrated on the chambers of the three monocot species, both because their similar shapes make comparisons among them more meaningful and because we used a very different technique to measure the volumes of willow capsules and were reluctant to treat the two techniques as if they were comparable.

Our two primary analytical tools were one-way ANOVA, with plant species as the grouping variable and using Sokal and Rohlf's (1987) method for determining the relative importance of within vs. between treatment variance; and linear regression, with spider or clutch mass as the independent variable. In both statistical contexts, our interest was in elucidating the sources of variation in capsule volume.

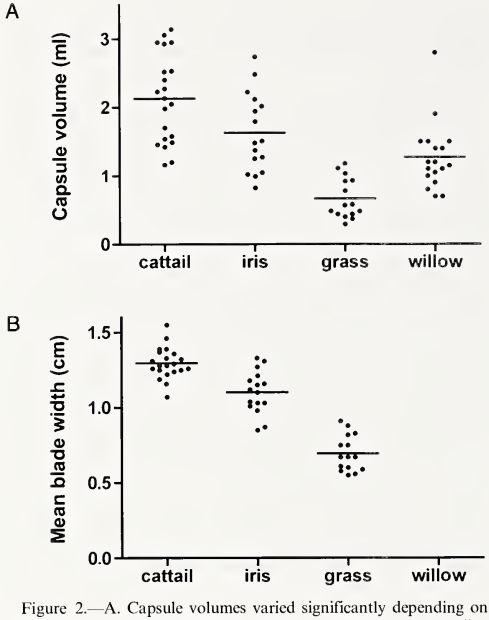


Figure 2.—A. Capsule volumes varied significantly depending on which plant leaves were used in construction. ANOVA was applied only to the monocots (willow capsule volumes were measured using different methods), and among them all pair-wise differences were significant. B. Mean blade widths, analyzed with ANOVA, also varied significantly among the three monocot plant species, and pair-wise tests were all significant. The most voluminous capsules were constructed with cattail blades and were so large in part because the mean blade width of cattails was large.

RESULTS

Among the monocots, capsule volume varied more than ten-fold, the smallest being a grass capsule with a volume of 0.29 ml and the largest being a 3.14-ml capsule made from a cattail leaf. The mean capsule volume (± SE) on cattails was 2.13 ± 0.14 ml, on iris 1.63 ± 0.14 ml, and on grass 0.67 ± 0.08 ml (Fig. 2A). ANOVA revealed that this variation was significantly associated with host plant species ($F_{2,39} = 32.04$, $P < 0.0001$), and Tukey's Multiple Comparison Test showed that all three pair-wise differences between the mean volumes were significant (cattail vs. iris, $P < 0.05$; cattail vs. grass, $P < 0.001$; iris vs. grass, $P < 0.001$). About 69.2% of the total variation (Fig. 2A) was attributable to differences among host plant species. Capsules constructed of willow, the only dicot, were intermediate in volume (1.27 ± 0.11 ml) between those on iris and those on grass (Fig. 2A).

An important component of capsule volume in monocots is the width of the blade where it becomes incorporated into the capsule, in this case measured at the two bends (Fig. 1). Given the significant differences in capsule volumes (above), it is unsurprising that blade widths were also significantly different, and with the same pattern (Fig. 2B). ANOVA showed that the differences among the mean widths of cattail (1.30 ± 0.02 cm), iris (1.10 ± 0.04 cm), and grass (0.70 ± 0.03 cm) were highly significant both in aggregate ($F_{2,49} = 108.4$, $P < 0.0001$) and in pair-wise tests ($P < 0.001$ for each).

Table 1.—Spiders constructed their capsules without regard to handedness on iris, grass, and willow, and without regard to which leaf surface became the external surface of the capsule on grass. In contrast, spiders on cattail built only clockwise capsules, and spiders on willow always left the upper surface of the leaf on the outside of the capsule. * Top and bottom surfaces were anatomically indistinguishable on the leaves of cattail and iris, rendering these distinctions not applicable.

	Proportion clockwise	*P*	Proportion with top surface outside*	*P*
Cattail	21/21	< 0.0001	NA	
Iris	6/16	0.227	NA	
Grass	7/13	0.500	6/13	0.500
Willow	11/18	0.240	18/18	< 0.0001

Capsules constructed on cattails were substantially higher above the water (112.4 ± 5.0 cm) than were capsules on iris (51.1 ± 2.1 cm), grass (59.9 ± 2.1 cm), or willow (53.8 ± 3.0). ANOVA showed these differences to be highly significant ($F_{3,65} = 76.1$, $P < 0.0001$), but in pair-wise tests, heights on iris, grass, and willow were indistinguishable from one another, and heights on cattails were significantly different from the others ($P < 0.001$ in each case).

Capsules on the four plant species also differed with respect to handedness, the direction of the two bends used to form the capsule (Fig. 1), and with respect to whether the top or the bottom surface of the leaf became the outside surface of the capsule (Table 1). Notably, spiders on cattail built only clockwise capsules, and spiders on willow always left the upper surface of the leaf on the outside of the capsule. Spiders on iris, grass, and willow showed no preference with respect to handedness, and spiders building on grass appeared to have no preference for one side of a leaf or the other as the outside surface of the capsule. We could not distinguish which side of a cattail or iris blade was top, so we did not score this attribute of capsules constructed on those two plant species.

In looking beyond host species for the sources of variation in capsule volume, we regressed capsule volume on spider mass, egg clutch mass, and pre-oviposition spider mass (the sum of spider and egg clutch masses). In doing this, we were aware that, because of its constituent components, pre-oviposition mass would be correlated with spider mass and with egg clutch mass. We also knew that many studies have found a strong direct effect of spider mass on egg clutch mass both among species (Marshall and Gittleman 1994; Nicholas et al. 2011) and within species (e.g., Killebrew & Ford 1985; Brown et al. 2003), a relationship that we also saw in our own data (Fig. 3; $r^2 = 0.221$, $F_{1,40} = 11.32$, $P = 0.0017$). Thus we knew that our several regressions were not independent of each other.

In our regression analysis (Fig. 4), capsule volumes (when pooled across plant species) were significantly influenced by spider mass, egg clutch mass, and pre-oviposition spider mass ($P = 0.050$, 0.023, 0.012, respectively). The strongest relationship was between pre-oviposition spider mass and capsule volume ($F_{1,40} = 6.90$; $r^2 = 0.15$). When the data were

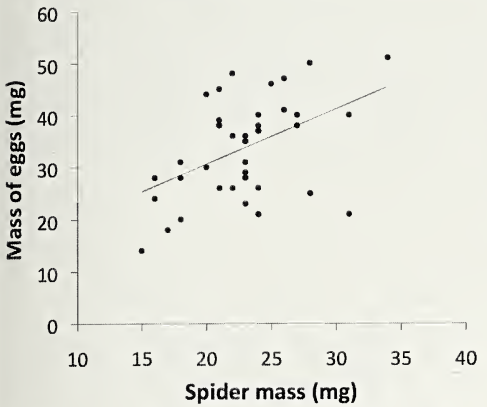


Figure 3.—About 22% of the variation in clutch mass was attributable to differences in spider mass ($r^2 = 0.221$, $F_{1,40} = 11.32$, $P = 0.0017$).

broken down by plant species, only among capsules built on cattails were there significant influences of the independent variables on capsule volume. And again there, the strongest relationship was between pre-oviposition spider mass and capsule volume ($F_{1,15} = 8.63$; $r^2 = 0.37$).

Despite a strong relationship between spider size and capsule volume, especially among capsules on cattails, we found no evidence that larger spiders were predisposed to build on cattails or, conversely, that smaller spiders chose to construct capsules on grass leaves (Fig. 5). ANOVA revealed that mean pre-oviposition spider mass did not vary significantly across the three monocot plant species ($F_{2,47} = 1.05$, $P = 0.358$).

DISCUSSION

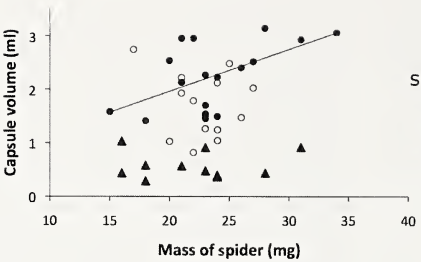
Many *C. riparia* construct their capsules on cattail, iris, or willow, despite the fact that grass blades, on which they can also construct capsules, are close by and in abundance. This suggests that suitable building sites were not a limiting resource in this area, but more importantly, it suggests that predisposition and choice could be involved. Choosing cattail, for example, means having the option to make a substantially larger capsule than could be constructed on a grass blade (Fig. 2), and that might well be advantageous for a large spider gravid with a large clutch of eggs. The data on pre-oviposition spider mass contradict that suggestion: the plant species on which a spider constructed a capsule was unrelated to the spider's size (Fig. 5). Moreover, at least for spiders that constructed capsules on iris or grass, the size of the spider appears not to have influenced the size of the capsule that it made (Fig. 4).

In contrast, we have strong evidence that spider size influenced the volume of capsules that were constructed on cattails: more than a third of the variation in capsule volume on cattails was attributable to the pre-oviposition masses of the spiders, with a doubling in spider size resulting in about a 20% increase in capsule volume (using the slope of the line in the bottom graph in Fig. 4). We also now know (Table 1) that these spiders always bend willow leaves to fashion a capsule that has the upper surface of the leaf to the outside, and that when they build on a cattail blade they always turn the blade in a clockwise direction (according to the convention we have adopted: see Fig. 1). What do these three observations — 1) the spider's scaling of the volume of its capsule to the spider's own mass, 2) the spider's consistent attention to willow leaf surface properties, and 3) the spider's proclivity for clockwise handedness when building on cattail but not elsewhere — imply about the kinds of pressures a gravid female *C. riparia* faces? We consider these questions in order below.

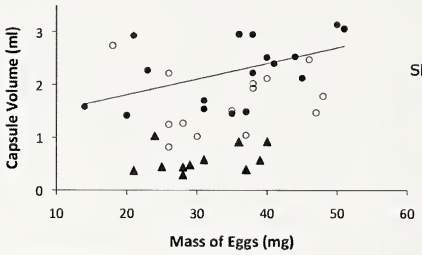
Scaling capsule volume to spider mass.—Although architectural feats are not often analyzed in this way, it is very clear that many spiders know how to measure, and that they adjust the sizes of their structures to fit their needs. As araneids grow, for example, so do their webs, presumably both because they are able to build larger webs and because they have greater metabolic needs, and larger webs intersect larger numbers of prey (Eberhard 1990). Similarly, burrowing wolf spiders increase the diameter of their burrows as they grow (Carrel 2003), and desert widow spiders increase a number of web and retreat dimensions as the spiders grow (Lubin et al. 1991). In that context, the spider size/capsule size relationship in *C. riparia*, and the spider's implied ability to measure, are not surprising.

Moreover, the scaling of capsule volume to spider mass makes sense from a biomechanical perspective. First, capsule volume must be sufficient to enclose both the spider and its eggs as separate entities (Fig. 6: not just as the single gravid organism that constructed and first inhabited the capsule) and to allow for the spider's movements while sealing the capsule from the inside and while laying eggs. Second, if predation by animals that would breach the capsule by cutting through the plant material (Fig. 6: as opposed to tearing the silk where two leaf edges meet) is important, then larger capsule size is better because, at least in the monocots, the leaf blade gets thicker as it gets wider. Third, a larger spider's size means that it can exert greater forces and, perhaps, can expend more energy during capsule construction (R.B. Suter et al. unpublished data) than can a smaller spider, allowing it to bend wider and stiffer leaves and thereby enclose more volume.

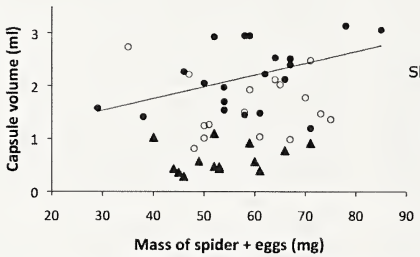
If larger leaf-curling sac spiders are able to construct larger capsules, and if there are advantages to doing so, why was the scaling of capsule volume to spider mass only observed when the spiders build on cattails? Statistically, this is not a trivial dichotomy: on cattails, the relationship is robust, explaining more than 36% of the variation in capsule volume; on iris and grass, the relationship is insignificant, and not just marginally so (Fig. 4). The leaves of the grass, *Calamagrostis* sp., at their widest, where the spiders bend them to make capsules, are about half the width of the part of the blades of cattails that the spiders use (Fig. 2B). That means that, were a spider to try to make a more voluminous capsule on a blade of grass, it would have to do so by elongating the capsule; but that would not appreciably improve the spiders maneuverability inside the narrow capsule and, because the spider was already doing its construction at the widest part of the blade, the resulting long capsule would not be more resistant to the depredations of gnawing animals.



	Cattail	Iris	Grass	Pooled
Slope	0.227	-0.091	0.114	0.147
r^2	0.275	0.030	0.112	0.093
F	5.788	0.376	1.137	4.095
DF	1,15	1,12	1,9	1,40
P	0.030	0.551	0.314	0.050



	Cattail	Iris	Grass	Pooled
Slope	0.030	0.005	0.012	0.033
r^2	0.252	0.006	0.091	0.123
F	5.043	0.075	0.900	5.583
DF	1,15	1,12	1,9	1,40
P	0.040	0.788	0.368	0.023



	Cattail	Iris	Grass	Pooled
Slope	0.101	0.003	0.036	0.065
r^2	0.365	0.001	0.077	0.147
F	8.629	0.009	0.754	6.896
DF	1,15	1,12	1,9	1,40
P	0.010	0.927	0.408	0.012

Figure 4.— On cattail (filled circles; regression line), capsule volume varied significantly with spider mass, egg clutch mass and pre-oviposition spider mass (the sum of spider mass and clutch mass). Those relationships were not found with capsules built on iris (open circles) or grass (triangles). On cattail, the strongest relationship was between capsule volume and pre-oviposition spider mass, where differences in mass accounted for 36.5% of the variation in capsule volume.

That line of reasoning, which provides a tenable explanation for the constrained volumes of capsules on grass blades, irrespective of spider size, does not serve well for capsules on iris blades. These blades, though about 15% narrower than cattail blades, are of much the same shape and share with cattail blades the property of becoming thicker and stiffer as one moves down the blade from the tip. Thus, as they do on cattails, larger spiders could make more voluminous capsules on iris, but they do not. We do not currently have a way to explain why the scaling of capsule volume to spider mass does not happen on iris.

Bending willow leaves to put the top surface outside.—When a spider constructs its capsule using a willow leaf, it does so by bending the leaf toward its lower side, resulting in a chamber that has the lower surface of the leaf on the inside and the upper surface of the leaf on the outside (Table 1). The willow leaves used by spiders at our study site were strongly asymmetrical, with a relatively smooth, shiny, dark upper surface that was devoid of stomata, and a much more textured and lighter lower surface with vascular tissue in relief and many stomata (R.B. Suter unpublished data). The presence of gas exchange pores, the stomata, consistently on the interior faces of the capsule walls suggests that the consequent differences in humidity and possibly respiratory gases are important to the spiders.

Desiccation is surely a problem for spiders and their eggs (Gillespie 1987; Hieber 1992; DeVito & Formanowicz 2003), and probably led to the evolution of known behavioral and architectural solutions (Humphreys 1975; Suter et al. 1987; DeVito & Formanowicz 2003). We presume that capsule construction by *C. riparia* also serves to reduce desiccation, both of the spider and of its egg clutch. Part of that function,

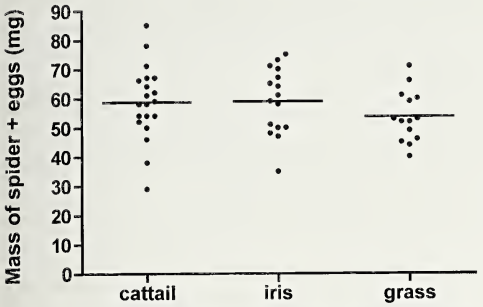


Figure 5.—Pre-oviposition spider masses did not vary significantly depending on which plant leaves were used in construction ($F_{2,47}$ = 1.05, P = 0.358), an indication that a spider's choice of one plant species over another was not biased by the spider's mass.

the provision of shelter from the forced convection of winds and from insolation, would be provided even if the sides of the capsules were made of willow leaves that had no stomata. But the presence of stomata on the inside means that water vapor lost from the plant during normal transpiration would dwell inside the capsule until it diffused outward through the spaces between the silk-joined edges of the leaves, thus keeping the relative humidity of the capsule's interior at close to 100%.

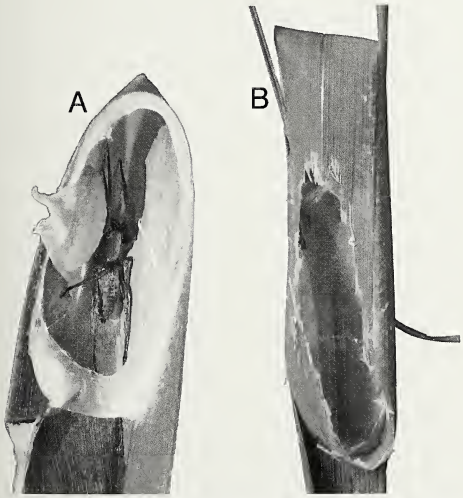


Figure 6.—Two opened capsules, shown approximately to scale. A. An inhabited capsule, opened by the authors, shows the female *C. riparia* with its egg mass and portrays the relationship of their size to the volume of the capsule. B. An empty capsule showing damage probably caused by a predator that gained access to the spider and eggs by tearing through the plant material rather than by separating the grass blades at a silk-closed seam.

This hypothesis is mildly supported by the observation that the spiders do not favor one side or the other of the grass (Table 1) because the species of *Calamagrostis* on which we found the spider capsules was amphistomatal, with stomata on both surfaces of each blade (R.B. Suter unpublished data), as is usual in this grass genus (Ma et al. 2005).

We have no direct evidence concerning the relative humidity inside the capsules on any of the host plants, so a test of our contention that the particular structure of willow capsules functions to boost interior humidity must await further study. Three alternative hypotheses about the topside-outside construction of willow capsules relate to the fact that the underside of the willow leaf is much more reflective than the top side: building a capsule with the underside inside a) makes the capsule less conspicuous to visually orienting predators; b) causes the capsule to absorb more solar energy under sunny conditions, thereby raising internal temperature; and c) keeps the more photosynthetic layers of the leaf exposed, thereby possibly inhibiting abscission and prolonging the life of the leaf (Taylor & Whitelaw 2001).

Bending cattail blades clockwise.—Our data on cattail capsules show a striking and highly significant handedness: all of the spider-bearing capsules on cattails were constructed by bending the blade clockwise (Table 1), whereas on the other three plant species there was no evidence of handedness. Asymmetries of this sort, in which an animal's morphology or behavior is in some way chiral, have received much attention in recent years, particularly as researchers have demonstrated that some chirality at the level of gross morphology, brain laterality, and behavior, is a consequence of chirality at the molecular and early developmental levels (Levin & Palmer 2007; Okumura et al. 2008; Davison et al. 2009). In the current case, we do not know whether the asymmetry resides in the gravid spider or in the cattail leaf.

Our working hypothesis is that the amphistomatal (Kaul 1974) cattail leaf is asymmetrical with respect to how easily it bends — that it is somewhat less energetically costly for the spider to bend it with a clockwise bias than with a counterclockwise bias, and that this difference is large enough to matter in the evolutionary calculus leading to an optimum architecture. Support for this hypothesis could come from measurements of the work required to bend cattails clockwise vs. counterclockwise, and that study is underway. To make good sense, however, that support would have to be paired with similar measurements of iris blades, because they are superficially nearly identical to cattail blades but are not treated as identical by the spiders (Table 1).

Despite the structural simplicity of the elegant capsules built by *C. riparia*, our analyses of their sizes and their locations revealed substantial complexity. The gravid spiders that constructed the capsules did so not only on narrow-bladed grass leaves and on the broader blades of iris and cattails but also on willow leaves. Given this variety of construction sites, it is not surprising that capsule volume varied widely (the smallest had a tenth of the volume of the largest), but it is surprising that only on cattails was there a significant relationship between spider size and capsule volume. Capsules found on cattail blades were also unusual in having been consistently constructed by bending the blades clockwise, while no chiral preference was seen in capsules built on the

other three plant species. Finally, the spiders always folded a willow leaf so that its stomata-bearing surface faced, and could perhaps modify or modulate, the enclosed atmosphere of the capsule.

This account is only a beginning. We are currently conducting four related studies: measuring the energetics of capsule construction, testing the spiders for preferences among the available plant species, analyzing the ways in which the microenvironment inside a capsule differs from external conditions, and seeking the source(s) of the chirality in capsule construction on cattails.

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Gait characteristics of two fast-running spider species (*Hololena adnexa* and *Hololena curta*), including an aerial phase (Araneae: Agelenidae)

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Abstract. Funnel-web spinning spiders of the genus *Hololena* are capable of fast movements in a horizontal plane across a variety of challenging surfaces. We used two species, *H. curta* (McCook 1894) and *H. adnexa* (Chamberlin & Gertsch 1929), in experiments designed to reveal how they achieve remarkable speeds, occasionally exceeding 70 body lengths (~50 cm) per second. In high-speed recordings we found that spiders used their legs in alternating sets of four, distributed in staggered pairs along the body axis, resulting in an alternating-tetrapod gait. Increases in speed showed positive linear relationships with both frequency and stride length. There were also inverse, linear relationships in both species between speed and duty factor, meaning that increases in speed are associated with a decrease in the relative amount of time spent by the legs on the ground during each full leg cycle. By examining their duty factor vs. speed regressions, we found that spiders of both species were capable of aerial phases during high-speed running, with the transitional speed occurring at an average of 54 body lengths per second. We conclude that further experimentation with high-speed spiders and insects will likely show that a variety of species exhibits dynamically stable locomotion, including aerial phases.

Keywords: Kinematics, locomotion, duty factor

Running ability can save an animal from predation, help procure its next meal or help it find and interact with a prospective mate. Neural control, kinematics, dynamics and energetics of locomotion have all been studied extensively in a variety of arthropod taxa, including cockroaches (Full & Ahn 1995; Full & Tu 1990; Jindrich & Full 2002), flying insects (e.g., Dickinson & Götz 1993; Lehmann & Dickinson 1998), stick insects (e.g., Frantsevich & Cruse 1997) and aquatic beetles (Nachtigall 1980). These cover a range of locomotion strategies and underlying mechanisms, including fast running, slow walking, flying and swimming (Dickinson et al. 2000). Information on spider biomechanics is largely limited to the energetics of courtship and walking and to the running kinematics of large mygalomorph spiders such as tarantulas (Wilson 1967; Shillington & Peterson 2002) and other spiders (Lighton & Gillespie 1989; Watson & Lighton 1994). Additional research has concentrated on the contribution of spring- and hydraulically-based mechanisms and related functional morphology to movement in a variety of arachnids, as well as general jumping ability (Parry & Brown 1959; Anderson & Prestwich 1975; Sensenig & Shultz 2003; Shultz 1989; Suter & Gruenwald 2000; Weihmann et al. 2010). Other mechanical studies focus on abilities unusual for spiders or arthropods in general, such as swimming or water-walking ability (Shultz 1987; Suter et al. 1997; Suter et al. 2003). Still others highlight the mechanisms of spiders' web construction and use (Opell 1994,1996; Naftilan 1999; Foelix 1996).

Despite the manifest importance of speed for small predatory animals, the gait kinematics of fast-running spiders has not been treated thoroughly in the literature. One exception is Foelix's (1996) description of locomotion of spiders as a pair of alternating tetrapods that become more synchronous (tighter temporal linkage between all four legs of a single tetrapod) at higher speeds. There are few published data and no model put forth to support his assertion; however, it provides a testable hypothesis for evaluation. Because of the differences between and difficulty of comparing gait patterns across and even within different animal taxa (Blickhan et al. 1993), it is important to characterize a wide range of animals' kinematics to understand the general principles that can be used to model terrestrial locomotion.

Speed and stability.—With their plurality of legs, arthropods are notably adept at movement by means of careful placement of legs at slow speeds. Various species can climb obstacles, scale inclines and vertical surfaces (reviewed in Delcomyn 1985) and even bridge gaps exceeding their own body lengths via careful leg extension (Blaesing & Cruse 2004). Such behavior can be termed 'static stability.' At no point would the animal 'fall down' if motion were somehow to be temporarily suspended. But running is better modeled as a "spring-loaded inverted pendulum" (or SLIP; Alexander 1988, 2003; Holmes et al. 2006). This means that at the lowest point in an individual step, the leg acts as a spring or a bouncing ball, storing and releasing energy such that at the top of each step (when the leg is off the ground, or in swing phase), both kinetic and potential energy are maximized and in phase. This results in aerial phases in many animals, where a normal step-cycle includes a portion where there is no leg contact with the ground. These gaits require dynamic stability, for which kinetic energy both helps prevent falling, and, along with forward momentum, allows fast-moving animals to bridge gaps in the substrate that would impede slower animals relying on static stability (Ferris et al. 1998; Daley et al. 2006; Spagna et al. 2007).

Recent comparative work has been performed to characterize foot-surface interactions on challenging surfaces (Spagna et al. 2007) using spiders (*Hololena adnexa* [Chamberlin & Gertsch 1929]) along with insects, crustaceans, and robots. This research demonstrated that the spiders were capable of fast, stable locomotion (≥ 50 cm/s, 70 body lengths/s) on mesh surfaces with varying probabilities of contact. The purpose of the present study is to characterize more thoroughly the gait characteristics of two fast moving, horizontally oriented spiders, *Hololena adnexa* and *Hololena*

curta (McCook 1894), on flat surfaces. This is done by examining gait parameters, including speed, duty factor and same-side limb phase for these species. This will provide a description of gait in fast-moving spider species and test Foelix's (1996) hypothesis of a positive correlation between running speed and tetrapod synchrony. This work also provides data from fast-running chelicerates for comparative work on kinematics and dynamics of terrestrial arthropods.

Terms used.—The typical gait of running spiders uses eight legs in two groups of four, alternating along the spider's anterior-posterior axis; for example, left legs I and III would be on the ground at the same time as right legs II and IV, while the other four legs would be off the ground, in motion (Foelix 1996). It is analogous to the 'alternating tripod' gait of fast-moving cockroaches and other insects (Full & Tu 1990), with an additional pair of legs behaving as additions to the tripods. For these reasons, this gait is referred to as an alternating tetrapod gait, with tetrapod referring to a set of four synchronous legs, not to a four-legged animal.

Swing phase is defined as the portion of a step cycle in which the leg (or the entire tetrapod) is moving toward another point of contact with the ground. Stance phase is the portion of a step cycle in which the leg or legs in question are in contact with the ground and providing a point upon which the animal can pivot the limb, or generate an acceleration force. Same-side limb phase is the mean fraction of a full step-cycle (stance phase plus swing phase) that passes before the opposite tetrapod touches down. Duty factor is defined as the mean amount of time each tetrapod spends in stance phase, normalized by the length of the full step cycle. The same-side limb phase convention is adapted from the study of quadrupedal animals (Hildebrand 1976), but instead of referring to the relative phasing of single legs, it describes relative phasing of the pairs of legs (I and III, II and IV) that move in phase in the alternating tetrapod gait.

METHODS

Samples of *Hololena adnexa* were collected from the shrubbery around the campus of the University of California, Berkeley, Alameda County, California, and *Hololena curta* specimens were collected from various locations in Riverside, Riverside County, California. The spiders were housed in 9-dram vials and fed small crickets after experimental runs. The spiders were made to leave the vials by gentle prodding with a pipe cleaner, which prompted them to disengage from the webbing in the container and drop into the filming arena. The drop (~10–20 cm total) provoked an escape response, causing the animals to run across the filming arena.

Spiders were filmed with two high-speed digital video cameras set orthogonally to each other (one from the top; the other from the side) (Redlake Imaging MotionScopes) with lenses of variable focal length (10–25 mm) at 500 or 1000 frames per second. More than two duplicate runs were not allowed during a single experimental session to prevent individuals from fatiguing (Foelix 1996), so that each spider experienced only one or two runs per day. After experimental trials, the spider was collected and placed back into its vial and allowed to recover overnight. Following the experiments, spiders were weighed, measured (cephalothorax plus abdomen length) and vouchers stored at −20° C at William Paterson University.

To calculate gait parameters, we measured speed by counting the number of frames (at 1 or 2 ms per frame, depending on recording speed) required to cross the test surface. Gait analysis was performed by mapping gait phase for each leg manually on graph paper. Placing phase graphs for all eight legs on the same set of axes allowed estimates of the relative phase of and overlap between the animals' leg placement. Duty factor (the fraction of time spent by a single tetrapod in contact with the ground during a full step-cycle, including both stance and swing phases) for each run was calculated by dividing the number of frames in stance phase (for both tetrapods) by the mean sum of frames in stance and frames in swing for leg pair I. Leg pair I was chosen arbitrarily, as legs are similar in length in this family and, with a symmetrical gait, should spend a similar amount of time on the ground.

Linear regression was performed to determine the relationship between speed and duty factor, speed and stride length, speed and stride frequency, and speed and synchrony factor. Comparisons were only made between runs with at least one complete stance phase of a tetrapod, so that the full swing/stance cycle for one tetrapod could be calculated. Stance phase was averaged when multiple tetrapod stances were recorded from a single run. Runs were disqualified where one or more legs were not in the frame long enough to provide data for tetrapod characterization or comparison.

Period was measured as the total time in stance plus swing for leg I, and frequency as its inverse. Stride length was measured as total distance between the surface contact points for leg I in the first two visible stance-phases.

Tetrapod synchrony was calculated by dividing the number of frames in which all four legs in a single tetrapod were in stance phase, from the total number of frames in stance by any of the legs in that tetrapod. This calculation gave a fractional factor between 1, representing perfect synchrony between all four legs in a tetrapod, and 0, for a situation in which no frames contained all four legs in stance phase. To test the Foelix hypothesis that synchrony increases with speed, we plotted synchrony against speed and carried out regression analysis on the paired data from each spider. For an additional test of synchrony, we performed a linear regression between speed and same-side limb factor and then performed a regression of the residuals on speed.

Same-side limb phase was calculated as the mean point during limb phase of leg I (as above, the sum of all frames from beginning of stance through swing phase of leg I) at which any leg in the second tetrapod of legs made ground contact. Duty factor and leg phase were then plotted against each other and mapped on a Hildebrand Plot to characterize the type of gait or gaits used by the spiders (Hildebrand 1976, 1985).

Statistics.—All statistics were calculated using Minitab v. 13 (Minitab Inc., State College, Pennsylvania) and are expressed as (mean ± SD). Significance level for all statistical tests was set at $P < 0.05$, with a Bonferroni adjustment to account for all tests being performed twice (once for each species) resulting in a critical P of 0.025. Linear regression was used to calculate the relationships between duty factor and speed, stride length

and speed, frequency and speed, and leg synchrony and speed for each species. Regression slopes were subsequently tested for significant differences between species via ANCOVA.

RESULTS

Spiders ran using an alternating tetrapod gait, for which legs I and III on one side made contact with the ground in imperfect synchrony with legs II and IV on the other side, and vice versa, followed by the same pattern from the opposite side. A total of 28 runs was analyzed from 24 different individuals (1–3 runs per individual) of *Hololena adnexa*, and 19 runs from 5 individuals (1–6 runs per individual) from *Hololena curta*. Runs were included in the following analyses based on visual quality (staying in the focal plane of the cameras) and upon determination that the animal proceeded through at least 2 full step cycles while in frame without stopping or turning, to allow calculations of all the kinematic variables. Mean speeds of runs were 51.6 body lengths/s for *H. adnexa*, and 48.6 for *H. curta*, not significant (*t*-test assuming unequal variances, $P = 0.40$). Additionally, ANOVAs of running performance by individual were performed to determine whether pseudoreplication (multiple runs by individual specimens) was a significant factor, and no effect was found for either species ($P = 0.30$ and 0.29 for *H. adnexa* and *H. curta*, respectively).

Speed, duty factor and aerial phase.—Mean duty factors for runs by *Hololena adnexa* and *Hololena curta* were 0.53 ± 0.10 and 0.58 ± 0.13, respectively. A two-tailed *t*-test assuming unequal variances showed no significant difference between mean duty factors between the two species ($P = 0.13$). Duty factor was inversely correlated with speed for both species (Fig. 2). Linear regression analysis yielded relationships between duty factor and linear speed for the two species, with a slope of -96.88 and an intercept of 102.81 for *H. adnexa* and a slope of -61.89 and an intercept of 84.45 for *H. curta*; $P < 0.001$ for both species. ANCOVA revealed no significant effect ($P = 0.736$) on these regressions by species. A duty factor less than 0.5 indicates that the animal has an aerial phase in its leg placement patterns. Such duty factors were seen in both species, and happened in 39% (11 of 28) of *H. adnexa* runs and 32% (6 of 19) of *H. curta* runs (see Fig. 1 for an example of a step cycle in which all legs are visually clear of the surface for multiple frames). Aerial phases in the animals ranged from 1–10 ms in length.

Speed, frequency, stride length and tetrapod synchrony.—The spiders showed statistically significant linear regressions (Table 1) between both speed and stride length (normalized for body size of the individual), as well as between speed and stride frequency (total stance phase plus total swing phase; Figs. 3A, B). No abrupt transitions or changes in slope were seen in these distributions for either set of regressions. ANCOVA showed no significant differences between regressions of speed on stride frequency ($P = 0.236$) or stride length ($P = 0.160$) by species. The mean synchrony factors were 0.30 ± 0.17 for *H. adnexa* and 0.30 ± 0.12 for *H. curta*. Linear regression relating speed and synchrony factor were not significant for either species ($P = 0.06$ and 0.42, respectively; see Fig. 4 and Table 1). No abrupt transitions were seen in the amount of synchrony by either species. Testing the hypothesis another way, we examined same-side leg phase to see if the phasing between leg-pairs became more consistent with speed. There was no significant relationship between the magnitude of the residuals for leg-phasing for either species ($P = 0.50$ for *H. adnexa*; $P = 0.30$ for *H. curta*).

Gait description.—A modified Hildebrand Plot considering opposing tetrapods rather than mammalian leg pairs (Fig. 5) shows that the spiders use a symmetrical gait that can be described as a trot, with just over a third of them in a running trot with aerial phase (17 out of 47 runs, see above), while the rest maintain a walking trot, with both sets of four legs on the ground for a fraction of each step (Hildebrand 1976). All the data points from both species cluster around 40% same-side leg phase and 50% duty factor.

DISCUSSION

Transition to aerial phase.—Setting duty factor regression equations equal to 0.5, the point below which aerial phase occurs, and solving for speed gives normalized body speeds of 54.38 and 53.50 body lengths/sec for *Hololena adnexa* and *Hololena curta*, respectively. Although legs in swing phase are not in contact with the ground in most studies of gait, this relationship may not always occur in these spiders. While the front leg pairs (I, II and III) clearly swing free of the substrate, it is not always visually evident that the rearmost legs (pair IV) are in the air during the swing phase. Rather, at times they appear to be dragging the tarsi of the rear pair of legs, maintaining contact with the ground while pulling them to their next foothold, so that their swing phase more closely resembles a slide or shuffle in its early stages. *Hololena* spiders, like other spiders in the family Agelenidae (C.L. Koch 1837), have large setae extending at a ~70° angle from the leg axis, and with the tarsus positioned parallel to the ground, these hairs may still contact the surface while the shaft of the tarsus is above the surface. However, they bend easily toward the leg axis, allowing the leg to be dragged past obstacles without being impeded (Spagna et al. 2007). This strategy of shuffling the rear legs may provide added stability, though such a shuffling gait does not appear to be addressed or modeled in the kinematic literature of arthropods. The spiders' dragline silk, which is sometimes but not always tacked down to the substrate during runs, may also pull down or otherwise orient their abdomen or rear legs, possibly limiting their ability to lift their rear legs clear of the substrate in the early stages of swing phase. The production of dragline was not controlled in these experiments. Although aerial phases in gait have been reported for spiders galloping on the surface of water (Gorb & Barth 1994; Suter & Wildman 1999; Stratton et al. 2004), this study appears to be the first report of aerial phases achieved by a spider in a purely terrestrial context.

Speed and synchrony.—The hypothesis of increased synchrony between legs within the two alternating tetrapods (after Foelix 1996) at increased speed is plausible, given subjective viewings of the high-speed video of spider runs. However, it is not supported by the data presented here, since a statistically significant relationship between speed and leg synchrony is not seen in either species. The raw number of frames in which individual legs appear at least partially out of phase with the rest of a tetrapod is clearly greater in the slower runs of both species, but normalization of these measurements by duration of stance phase reduces that relationship to insignificance. It

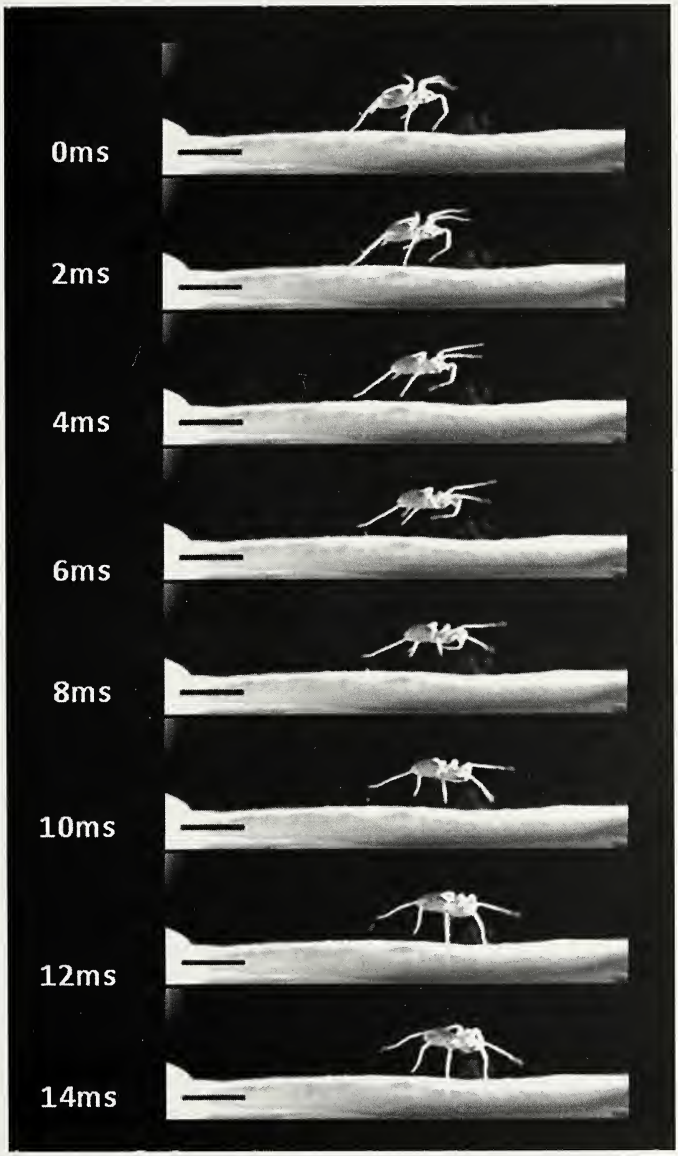

Figure 1.—Sequence of frames (alternating frames filmed at 1000 f/s) showing aerial phase achieved by a specimen of *Hololena curta.*

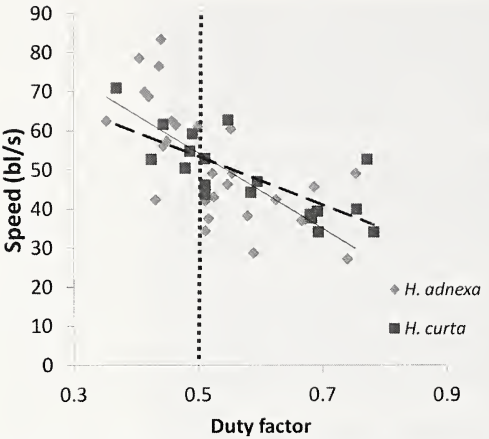

Figure 2.—Negative linear relationships between speed (normalized by body size) and duty factor for two species of grass-spider (*Hololena adnexa* = solid line, *Hololena curta* = hatched line). Points with duty factors of less than 0.5 (left of dotted line) represent runs where an aerial phase was indicated by the kinematic data.

appears that any reduction in variation at increasing speeds is proportional to the reduction in the duty factor and length of stance phase at increasing speeds.

Gait transitions.—There are several ways to characterize animal gaits, but it is not always simple to determine with certainty whether a movement is a walk, a run, or some intermediate gait (see Hutchinson et al 2003; Ahn et al. 2004). One rather obvious method, dating back to the early days of motion photography (e.g., Muybridge 1887), is the shift from a gait where at least one leg is in contact with the ground, to one where all legs are in swing phase— the shift to aerial phase. Aerial phases are relevant to the extent that they may represent a shift in or contribute to speed, energy efficiency, or stability.

The aerial phase is rare in arthropods (Blickhan et al. 1993), but a version of it is seen in the *Hololena* spiders characterized here. Other methods of categorizing running gait rely on changes in the stride frequency and stride length relative to speed. Blickhan & Full (1987) showed that while running, the ghost crab has two running regimes: a slow run for which stride frequency increases linearly with speed while stride length remains stable, and a fast run for which speed increases are associated with increases in stride length. This study, by contrast, showed increases in both stride length and stride frequency contributing to increase speed linearly across a range of speeds, including those for which the spiders show aerial phases. This means that obvious shifts (changes in slope or intercept of regression lines) in gait regime are not apparent with respect to speed. These data may also represent these spiders running in a narrow subset of the range of speeds possible for them. A treadmill experiment at speeds chosen by the experimenter could reveal frequency and stride-length transitions analogous to those characterized in ghost crabs, or some other type of transition at extremes of the speed spectrum not seen in this data set.

These analyses did not include force measurements or tracking of the animals' center of mass in three dimensions, so any discussion of the dynamics of stable running, which have been used in many studies to combine kinematics and kinetics (Blickhan & Full 1987; Blickhan et al. 1993; Full & Tu 1990), must remain largely speculative. However, the existence of the aerial phase without any obvious gait transitions suggests strongly that the animals must be dynamically stable, rather than statically stable, while executing a steady forward run (Ting et al. 1994). Without obvious transition points, we make the conservative assumption that throughout the range of speeds tested, the dynamics contributing to stability, such as phasing of kinetic and potential energies, remain the same. The smooth transition to the aerial phase with respect to speed and other gait parameters suggests that the gait being used is consistent. The shift is thus minor, and the consistency likely contributes to the dynamic stability of the animals during the entire range of runs studied, including those with the visible aerial phase, but also, and perhaps more importantly, during slower runs.

Without associated physiological data such as V_{O2} measurements (Anderson & Prestwich 1982, 1986), this study cannot address the question of changes in physiological efficiency of movement with transition to an aerial phase, or the spiders' use of dynamically stable locomotion across the full range of motion, but it does open the possibilities of future work in these areas. A reasonable hypothesis, given the linear appearance of the present data, is that if there is a transition in terms of physiological efficiency, it may occur below the range of speeds studied here.

Other taxa.—Although the Agelenidae, including the spiders tested here, are noted for being fast runners among the spiders (Bristowe 1968), with so few kinematic data available, there certainly are other likely untested candidates

Table 1.—Statistical relationships between speed and gait parameters in *Hololena* species.

Regression — Species	*n*	r^2	Slope	Y-intercept	*P*
Speed / duty factor — *H. adnexa*	28	0.45	−96.88	102.82	< 0.0001
Speed / duty factor — *H. curta*	19	0.57	−61.89	84.45	0.0002
Speed / stride length — *H. adnexa*	28	0.53	26.19	−5.32	< 0.0001
Speed / stride length — *H. curta*	19	0.39	17.39	9.92	0.004
Speed / frequency — *H. adnexa*	28	0.64	2.60	−8.40	< 0.0001
Speed / frequency — *H. curta*	18	0.13	1.20	20.52	0.15 (n.s.)
Speed / synchrony — *H. adnexa*	27	0.13	30.96	42.75	0.06 (n.s.)
Speed / synchrony — *H. curta*	19	0.04	−16.18	53.41	0.43 (n.s.)

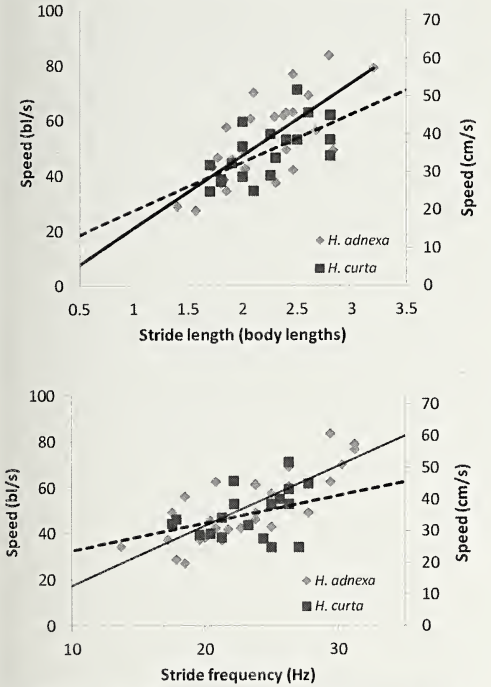

Figure 3.—Top panel: scatterplot and regressions for speed vs. stride length (relative to body length). *Hololena adnexa* runs are represented by diamonds and solid regression line; *Hololena curta* runs represented by squares and hatched regression line. Bottom panel: scatterplot and regressions for stride frequency (Hz) versus speed, with markers using the same conventions as top panel. Secondary axes show raw speeds estimated using the mean carapace plus abdomen length of 7.3 mm.

for the fastest spider. The large range of spider morphologies and running strategies makes these taxa a rich area for study. Spiders with laterigrade (sideways) leg orientations or gaits, such as those in the Thomisidae, Sparassidae and Selenopidae would provide interesting comparisons to both the spiders with more standard leg orientations and to the sideways-running ghost crabs (*Ocypode quadrata*), which are the best-characterized and fastest of eight-legged running animals (Blickhan & Full 1987; Blickhan et al. 1993). Other spiders and arachnids, particularly cursorial hunters such as Lycosidae (wolf spiders) and the Solifugae (wind-scorpions) appear to achieve extremely high speeds, though they have never been rigorously measured and documented. Spiders such as orb-web-weavers that forage in vertically oriented webs may also provide a useful counterpoint to these successful runners, as may the heavier, slower-moving Theraphosidae, or tarantulas (Wilson 1967).

From an evolutionary and comparative viewpoint, arachnids represent the terrestrial branch of a lineage, the

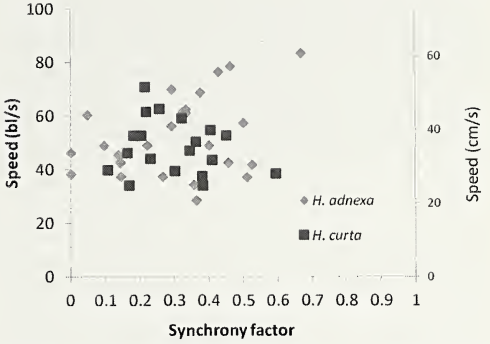

Figure 4.—Scatterplots of synchrony factor (normalized fraction of stride overlap between legs in the same tetrapod, see text) versus speed for *Hololena adnexa* (diamonds) and *Hololena curta* (squares). Regression lines (not shown) are not statistically significant at $P < 0.05$.

chelicerates, which diverged from the rest of the arthropods in the ocean at least 550 million years ago, approximately 100 million years before the invasion of terrestrial environments by any animals (Briggs et al. 1993). Thus, similar adaptations specific to terrestrial running behavior that have occurred in both insects and spiders can be considered the result of convergent evolution.

The family Agelenidae (consisting of over 1000 species if Coelotinae are included in the family, following Miller et al. 2010) and many of their relatives have a lifestyle dependent on foraging on irregular substrates such as shrubs and grasses (Roth & Brame 1972; Spagna & Gillespie 2008) and have a high vulnerability to predation and parasitism (Tanaka 1992). Therefore, the ability to escape quickly via a dynamically stable run requiring minimal nervous feedback (Spagna et al. 2007) is an adaptive hypothesis that should be further tested.

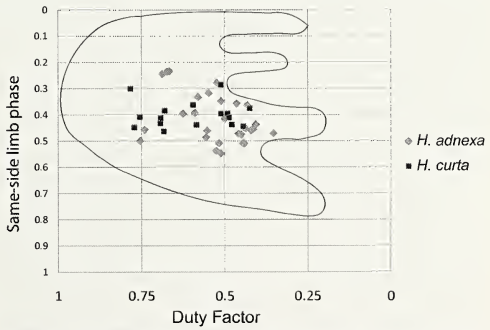

Figure 5.—Hildebrand plot of gait parameters (duty factor and same-side limb phase) used to determine animal gaits. Majority of points fall in range of medium to fast trotting gaits (third 'finger' from the top). Outline represents range of gaits of 156 genera of four-legged animals (Hildebrand 1989).

This work on *Hololena* spiders provides a starting point for comparing gait kinematics both within the Araneae and between spiders, other arachnids, and other fast-running arthropods. Such comparative studies seem likely to show that dynamically stable, fast-running behavior with an aerial phase occurs in other spider or arthropod lineages, making such gaits more common and providing a richer understanding of the evolution of running in arthropods with multiple leg-pairs.

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Two new species of Manaosbiidae (Opiliones: Laniatores) from Panama, with comments on interspecific variation in penis morphology

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Abstract. In Central America, the family Manaosbiidae is recorded only from Panama and Costa Rica. Four species occur in this region: *Barrona williamsi* Goodnight & Goodnight 1942, *Bugabitia triacantha* Roewer 1915, *Poassa limbata* Roewer 1943, and *Zygopachylus albomarginis* Chamberlin 1925. In this paper, we describe *Barrona felgenhaueri* new species (Coclé Province, Panama) and *Bugabitia akini* new species (Coclé Province, Panama) and report a new record for *B. williamsi* (Coclé Province, Panama). We used SEM to examine the penis morphology of *Barrona* Goodnight & Goodnight 1942 and the Caribbean species *Cranellus montgomeryi* Goodnight & Goodnight 1947 and *Rhopalocranaus albilineatus* Roewer 1932. We compared genital morphology of these species with published descriptions for Manaosbiidae from South America. With respect to genital morphology, we found that the most variable characters were the number and relative sizes of the setae that occur on the lateral margins of the ventral plate. Other features that exhibited interspecific variation included the shape of the ventral plate, the shape of the distal border of the ventral plate, and the shape and armature of the apex of the stylus.

Keywords: Central America, morphology, Neotropics, taxonomy

The Manaosbiidae is a member of the suborder Laniatores. It belongs to the superfamily Gonyleptoidea, a lineage that also includes the Cosmetidae, Cranaidae, and Gonyleptidae (Kury 2007). Recently, a phylogenetic analysis using molecular data (Giribet et al. 2009) supported the membership of Manaosbiidae within this clade. However, this study also indicated that Manaosbiidae is polyphyletic, at least with respect to the inclusion of the genus *Zygopachylus* Chamberlin. Manaosbiidae was initially recognized as the subfamily Manaosbiinae within the Gonyleptidae (Roewer 1943). Kury (1997) elevated the group to family status, refined the characters distinguishing the Manaosbiidae from the Cranaidae and Gonyleptidae, and provided diagnostic characters for the family.

Of the 47 species and 27 genera currently placed in the Manaosbiidae (Kury 2007), 12 species are known only from female holotypes (Kury 2003). The morphology of the penis, an important structure in modern taxonomic descriptions for Opiliones (Acosta et al. 2007), has only been described for seven species, all from South America (Šilhavý 1979; Kury 1997, 2007).

Currently, the Manaosbiidae has a geographic distribution that includes the Caribbean islands of Trinidad and St. Vincent, northern South America, and Central America (Kury 2003). Most species are small (3.5–10 mm in scutal length) and known from only relatively few records. This is due, at least in part, to undersampling or lack of sampling the leaf litter, a microhabitat in which they can be relatively abundant (Kury 2007). Little is known about the natural history of these harvestmen, although Townsend et al. (2008a) provided observations concerning activity, habitat use and geographic distribution for *Cranellus montgomeryi* Goodnight & Goodnight 1947a and *Rhopalocranaus albilineatus* Roewer 1932 on the Caribbean island of Trinidad. The two basal segments on tarsus I of males in most species are generally enlarged and frequently fused (Kury 2007). Observations of these segments with the aid of scanning electron microscopy (SEM) have revealed that these tarsal segments have numerous pore openings, which are hypothesized to be connected to packed clusters of exocrine glands that may function in intraspecific communication (Willemart et al. 2010). The only known species within the family from Central America that lacks the enlarged segments of tarsus I is *Zygopachylus albomarginis*. In this species, males construct and defend mud nests and mate with visiting females (Rodríquez & Guerrero 1976; Mora 1990). Following oviposition, the males remain in the nests and actively defend the eggs against ants and conspecifics (Mora 1990). Currently, four manaosbiid species are known from Central America, namely *Barrona williamsi* Goodnight & Goodnight 1942a (Panama), *Bugabitia triacantha* Roewer 1915 (Panama), *Zygopachylus albomarginis* Chamberlin 1925 (Panama) and *Poassa limbata* Roewer 1943 (Costa Rica). Each of these monotypic genera is endemic to Central America (Kury 2003). In this study, we describe two new species, *Barrona felgenhaueri* and *Bugabitia akini*. To provide greater insights into the phylogenetic relationships of manaosbiids, we used SEM to examine the penis morphology of *Barrona* and two Caribbean species (*Cranellus montgomeryi* and *Rhopalocranaus albilineatus*). We compared these observations to published descriptions of penis morphology for seven species from South America (Šilhavý 1979; Kury 1997, 2007).

METHODS

The specimens examined in this study are deposited in the American Museum of Natural History, New York, USA (AMNH); Senckenberg Museum, Frankfurt, Germany (SMF)

and the Museo de Invertebrados G.B. Fairchild de la Universidad de Panama, Panama City, Panama (MIUP). Specimens were examined and photographed with a Leica Zoom stereomicroscope. Digital images of specimens were processed and the body and leg segments were measured with the aid of a Leica image capturing system.

Adult males of *Barrona williamsi* were collected in the field by R. Miranda from Parque Summit, Panama Province, Panama in September 2009. We collected specimens of *Cranellus montgomeryi* and *Rhopalocranaus albilineatus* from the Central and Northern Ranges of Trinidad, West Indies in July 2006 and 2008. Penises were dissected and prepared for scanning electron microscopy (SEM). Specimens were dehydrated in a graded ethanol series, dried with hexamethyldisilizane, mounted on an aluminum stub with double stick tape, and sputter-coated with gold. Penises were examined and photographed with a Hitachi S-3000N SEM at an accelerating voltage of 15 kV in the Microscopy Center at the University of Louisiana at Lafayette, USA. In addition, the penis of an AMNH paratype of *B. felgenhaueri* was dissected and examined with a compound light microscope. This penis was placed into a genitalia vial in 70% ethanol and stored with the male. For diagnoses and descriptions, we employed terminology for morphological features of harvestmen used by Goodnight & Goodnight (1947), Kury (1997), Kury & Pinto-da-Rocha (2002), and Acosta et al. (2007).

SYSTEMATICS

Manaosbiidae Roewer 1943

Mitobatinae [part]: Simon 1879:226.
Prostygninae [part]: Roewer 1913:140; 1923:449; Mello-Leitão 1932:103; Goodnight & Goodnight 1942a:11.
Cranainae [part]: Roewer 1913:349; 1923:536; Mello-Leitão 1931:118; 1932:111; 1941:440; Roewer 1938:6; Goodnight & Goodnight 1942b:7; Soares & Soares 1948:583.
Hernandariinae [part]: Roewer 1913:460; 1923:582; Mello-Leitão 1932:129; Soares & Soares 1949:221.
Heterocranainae [part]: Roewer 1913:417; 1923:567.
Manaosbiinae: Roewer 1943:14, 56; Soares & Soares 1949:224.
Manausbiinae [misspelling]: Mello-Leitão 1949:12.
Stygnoleptinae [part]: H. Soares 1972:68.
Manaosbiidae: Kury 1997:3; Kury 2003:206; Kury 2007:209.

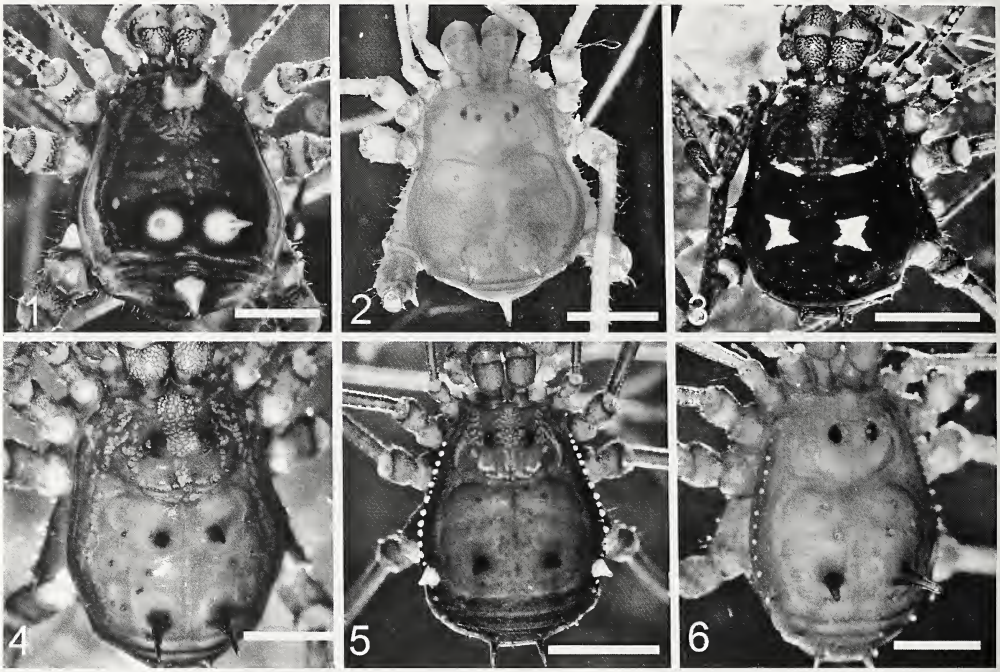
Emended diagnosis.—Gonyleptoidea with abdominal scute only slightly wider than carapace, ocularium small, without depression, unarmed or with 1–3 small or large spiniform tubercles; abdominal scutum unarmed or with paired tubercles, granular tubercles on area I generally smaller than the spiniform tubercles on area III; pedipalpus smooth, without strong armature on any segments; pedipalpal femur cylindrical; coxa IV barely visible above scute, dorsally covered with spiniform tubercles and armed with spiniform apical tubercle; trochanters I–III may have ectal tubercles; only basal segments of basitarsus I spindle-like in male; tarsi III–IV with a pair of smooth claws and occasionally sparse scapulae; ventral plate of penis rectangular elongate, with distal border substraight, concave, or with parabolic cleft, basal setae stout, slightly bent, median two pairs of setae of ventral plate dorsally located, distal setae flattened or strongly curved, but not helycoidal; stylus straight apex folded or papillate, glans exposed, without dorsal or ventral processes.

Distribution.—Brazil, Colombia, Costa Rica, Ecuador, Guyana, Panama, Peru, Suriname, Trinidad & Tobago, Venezuela, Windward Islands (St. Vincent and the Grenadines, Grenada).

Included genera.—*Azulamus* Roewer 1957, *Barrona* Goodnight and Goodnight 1942, *Belemnodes* Strand 1942, *Belemulus* Roewer 1932, *Bugabitia* Roewer 1915, *Camelianus* Roewer 1912, *Clavicranaus* Roewer 1915, *Cranellus* Roewer 1932, *Cucutacola* Mello-Leitão 1940, *Dibunostra* Roewer 1943, *Gonogotus* Roewer 1943, *Manaosbia* Roewer 1943, *Mazaruunius* Roewer 1943, *Meridia* Roewer 1913, *Paramicrocranaus* Soares 1970, *Pentacranaus* Roewer 1963, *Poecilocranaus* Roewer 1943, *Rhopalocranaus* Roewer 1913, *Rhopalocranellus* Roewer 1925, *Sanvicentia* Roewer 1943, *Saramacia* Roewer 1913, *Semostrus* Roewer 1943, *Syncranaus* Roewer 1913, *Tegyra* Sørensen 1932 and *Zygopachylus* Chamberlin 1925.

KEY TO THE MANAOSBIIDAE OF CENTRAL AMERICA

1. Second free tergite with single spiniform tubercle ..... (*Bugabitia*)...2
   Second free tergite with paired granular tubercles 3
2. Ocularium with paired spiniform tubercles; paired spiniform tubercles on abdominal scutal area III without smaller encircling granular tubercles; tarsal formula 6:15:6:8 ..... *Bugabitia akini* new species (Fig. 1)
   Ocularium with paired granular tubercles; paired spiniform tubercles on abdominal scutal area III encircled by smaller tubercles; tarsal formula 6:12:6:? ..... *Bugabitia triacantha* Roewer 1915 (Fig. 2)
3. Margins of abdominal scutum unarmed ..... (*Barrona*)...4
   Margins of abdominal scutum with single row of granular tubercles with terminal tubercle (adjacent to areas III or IV) enlarged 5
4. Scutum with 4 white patches; smaller patches on abdominal scutal area I, larger patches on area II; tarsal formula 6:12:6:6 ..... *Barrona felgenhaueri* new species (Fig. 3)
   Scutum without white patches; carapace black with lighter mottling; tarsal formula 6:16:6:7 ..... *Barrona williamsi* Goodnight & Goodnight 1942 (Fig. 4)
5. Terminal conical tubercle on margin of scutum much larger than other tubercles on scutal margin; anterior region of carapace with lighter mottling; more than 12 small tubercles on margins of abdominal scutum ..... *Zygopachylus albomarginis* 1925 Chamberlin (Fig. 5)
   Terminal tubercle on scutal margin only slightly larger than other tubercles on margin; anterior region of carapace without lighter mottling; less than 12 small tubercles on margins of abdominal scutum ..... *Poassa limbata* Roewer 1943 (Fig. 6)



Figures 1–6.—The Manaosbiidae of Central America: 1. *Bugabitia akmi*, new species, holotype, female; 2. *B. triacantha*, Roewer 1915, holotype, female; 3. *Barrona felgenhaueri*, new species, holotype, female; 4. *B. williamsi*, male from Colón Province, Panama; 5. *Zygopachylus albomarginis*, female from Barro Colorado Island, Panama; 6. *Poassa limbata*, (Roewer 1943), holotype, female. Scale bars = 2 mm.

Barrona Goodnight & Goodnight 1942

Barrona Goodnight & Goodnight 1942:11; Goodnight & Goodnight 1947:11; Soares et al. 1992:4; Kury 1997:4; Kury 2003:207.

Type species.—*Barrona williamsi* Goodnight & Goodnight 1942, by original designation.

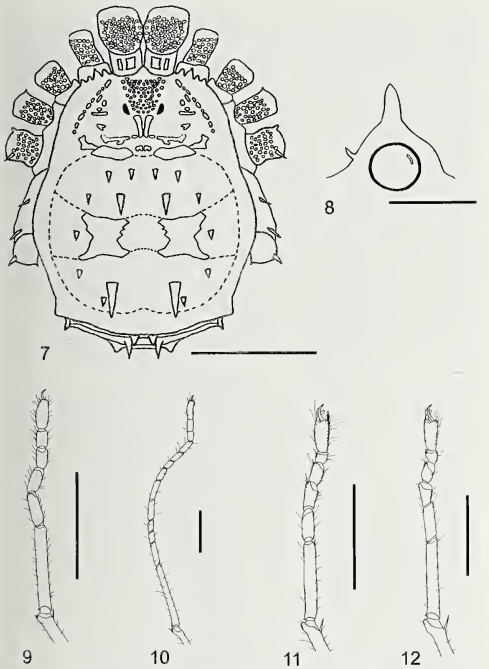
Emended diagnosis.—Anterior margin of carapace with 4–5 granular tubercles on each side. Eye mound with 2 granular tubercles on each side, anterior tubercle smaller. Abdominal scutal areas I and III with paired tubercles; spiniform tubercles on scutal area III much larger than granular tubercles on area I; area II unarmed, except for a few granular tubercles; areas IV–V unarmed and indistinct, margins of scutum unarmed. Lateral margins of scutum unarmed. Free tergites with paired granular tubercles, lateral edges with or without single tubercle on each side. Anal operculum with scattered granular tubercles. Pedipalpal femur and patella unarmed; tibia with 4 ectal (IiIi) and 4 or 5 mesal (IiIi or IiiIi) spines; tarsus with 4 ectal (IiIi) and 5 mesal (IiIi) spines. Coxa IV with 2 dorsal tubercles; posterior spiniform tubercle larger than anterior granular tubercle; femora III–IV with paired, dorsal apical tubercles; tarsal formula: 6:12–16:6:6–7; tarsal claws unpectinate. Color of scutum black to dark brown with or without white patches. Ventral plate of penis rectangular, elongate with concave distal margin; stylus unarmed, bent with a folded apex. Basitarsus I of male spindle-like; 2 basal segments enlarged.

***Barrona felgenhaueri*, new species**
(Figs. 3, 7–15)

Material examined.—PANAMA: *Coclé Province*: Holotype female, Parque Nacional General Division Omar Torrijos H., El Cope (08°49.2′80″N, 80°05′45.7″W), 23–28 February 2007, V. Townsend, A. Savitzky and J. Ray, collected by hand along hiking trails at night in montane rainforest (AMNH). Paratypes: 1 female, collected with holotype (AMNH); 1 male, same location, 1–4 November 1980, D. Mosley (MIUP).

Etymology.—This species is a patronym in honor of Bruce Felgenhauer who has made many contributions to the study of the morphology and natural history of tropical arthropods.

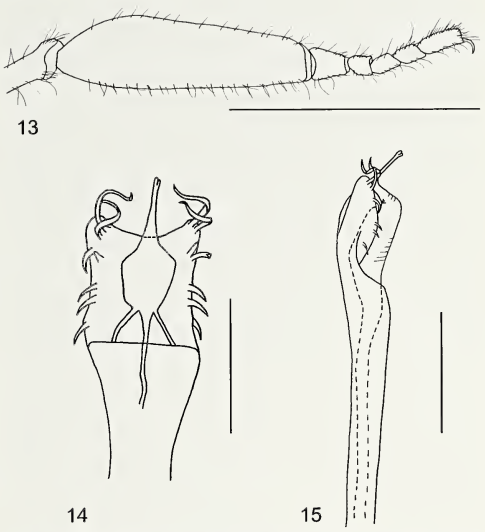
Diagnosis.—Dorsal scutum attenuate pyriform with scutal areas poorly defined, area I with 3 granular and 1 spiniform tubercle each side, area II with 1 granular tubercle and a large white patch each side, area III with 2 granular and 1 large spiniform tubercle each side, areas IV–V indistinct and unarmed (Fig. 7). Ocularium with large spiniform tubercle and a smaller anterior granular tubercle each side (Fig. 8). Anterior margin of carapace with 4 small granular tubercles



Figures 7–12.—*Barrona felgenhaueri*, new species, female, holotype: 7. Habitus, dorsal view; 8. Ocularium, lateral view; 9. Tarsus I, lateral view; 10. Tarsus II, lateral view; 11. Tarsus III, lateral view; 12. Tarsus IV, lateral view. Scale bars = 2 mm (Fig. 7); 0.3 mm (Fig. 8); 1 mm (Figs. 9–12).

on each side (Fig. 7). Cheliceral sockets of carapace shallow (Fig. 7). Cheliceral bulla smooth. Basal tarsal segments I of the male swollen and spindle-like (Fig. 13). Free tergite I with paired granular tubercles; II with paired spiniform tubercles and 1 granular tubercle on the margin each side; III with paired granular tubercles and 1 granular tubercle on margin of each side (Fig. 7). Femur and tibia IV straight. Tarsal formula 6:13:6:6. Tarsal claws III–IV unpectinate (Figs. 9–12). Penis: ventral plate with lateral borders straight and parallel, distal border slightly concave, uncleft; with third and fourth distal curved spines flattened; glans without dorsal or ventral process; stylus bent with folded apex (Figs. 14, 15).

Description.—*Female:* Measurements (paratype, in mm): dorsal scute length 4.17; cephalothorax length 1.35; mesotergum width 3.73; cephalothorax width 2.64; leg segments (length): trochanter I: 0.48; femur I: 2.88; patella I: 0.83; tibia I: 1.59; metatarsus I: 2.95; tarsus I: 2.38; total leg I: 11.11; trochanter II: 0.57; femur II: 6.15; patella II: 1.28; tibia II: 4.25; metatarsus II: 5.46; tarsus II: 5.26; total leg II: 22.97; trochanter III: 0.61; femur III: 4.58; patella III: 1.24; tibia III: 2.30; metatarsus III: 4.54; tarsus III: 2.35; total leg III: 15.62; trochanter IV: 0.61; femur IV: 6.12; patella IV: 1.39; tibia IV: 3.06; metatarsus IV: 6.61; tarsus IV: 3.14; total leg IV: 20.93.



Figures 13–15.—*Barrona felgenhaueri*, new species, male, paratype: 13. Tarsus I, lateral view; 14. Penis, dorsal view; 15. Penis, lateral view. Scale bars = 2 mm (Fig. 13); 250 μm (Figs. 14, 15).

Dorsum (Fig. 7): anterior margin of carapace with 4 granular tubercles on each side; eye mound with a spiniform tubercle and an anterior granular tubercle on each side (Fig. 8); abdominal scutum with 4 distinct areas; area I with paired larger granular tubercles and 6 smaller granular tubercles; area II with 2 granular tubercles; area III with paired spiniform tubercles and 4 granular tubercles; areas IV–V indistinct and smooth; granular tubercles in areas I–V bearing small spines; lateral margins of abdominal scutum without tubercles. Free tergite I with pair of granular tubercles; II with pair of median granular tubercles and 1 granular tubercle on the margin each side; III with pair of median granular tubercles and 1 granular tubercle on the margin each side; tubercles on free tergites similar in size and shape and bearing spines. Anal operculum with 8 granular tubercles bearing spines.

Venter: coxae I–III with 1–2 rows of granular tubercles bearing spines, IV with scattered granular tubercles bearing spines.

Chelicera: smooth with sparse setae.

Pedipalp: trochanter length: 0.51 mm; femur length: 1.47 mm; patella length: 0.93 mm; tibia length: 1.21 mm; tarsus length: 1.22; total length: 5.34; coxa with one ventral tubercle bearing a spine; trochanter with one mesal tubercle bearing a spine; femur and patella smooth; tibia ectal IiIi, mesal IiIi; tarsus ectal IiIi, mesal IiIi.

Legs (Figs. 9–12): coxa IV with 2 spiniform tubercles; trochanters with a retrolateral granular tubercle; femora I–II smooth, femora III–IV with dorsal, apical spine on retrolateral surface, patellae-tarsi I–IV smooth with sparse spines; tarsal formula: 6:12:6:6.

Color: dorsum dark brown-black, with paired white patches on scutal groove and paired white patches on scutal area II, posterior patches larger than anterior ones; trochanters, patellae and chelicerae darker than pedipalps and femora; metatarsi annulate.

Male: Measurements (in mm): dorsal scute length 4.39; cephalothorax length 1.57; mesotergum width 3.78; cephalothorax width 2.85; total length pedipalp: 5.65; total length leg I: 12.49; total length leg II: 24.34; total length leg III: 16.58; total length leg IV: 21.84. Leg I: similar to female with the exception that the 2 most basal segments are swollen (Fig. 13). Legs II–IV similar to female. Tarsal formula: 6:13:6:6.

Color: similar to female.

Genitalia (Figs. 14, 15): truncus long and slender; ventral plate elongate subrectanglar, tapering towards distal margin, with a distal border entire, slightly concave (Figs. 14); lateral borders with 5 straight + 2 recurved setae (Figs. 14, 15). Stylus straight with apex folded and unarmed (Fig. 14).

Habitat.—Specimens were collected from vegetation and spaces beneath logs from hiking trails in montane rainforest on a moderate slope. They were found after dark between 2100–2300 hr in the dry season during light to moderate periods of rainfall.

Barrona williamsi Goodnight & Goodnight 1942
(Figs. 4, 16–19)

Barrona williamsi Goodnight & Goodnight 1942:11, fig. 26; Goodnight & Goodnight 1947:11, figs. 1, 2; Soares et al. 1992:4; Kury 2003:207.

Material examined.—PANAMA: *Coclé Province*: Male, Parque Nacional General Division Omar Torrijos H., El Cope (08°49.2′80″N, 80°05′45.7″W), 23–28 February 2007, V. Townsend, A. Savitzky and J. Ray, captured by hand along trails at night in montane rainforest (AMNH); *Colón Province*: male, Parque Nacional Soberania (09°07′55.3″N, 79°43′14.2″W), 1983, L. Sorkin (AMNH); *Panama Province*: male, Parque Nacional Summit (09°03′41.08″N, 79°38′55.75″W), September 2009, R. Miranda, captured by hand beneath logs and rocks during the morning (AMNH).

Description.—*Male genitalia* (Figs. 16–19)*:* Truncus long and slender; ventral plate defined as an elongate subrectangle, tapering towards distal margin, with a distal border entire, slightly concave (Figs. 16, 17); lateral borders with 4 straight + 3 recurved setae (Fig. 18). Stylus straight with apex folded and unarmed (Fig. 19).

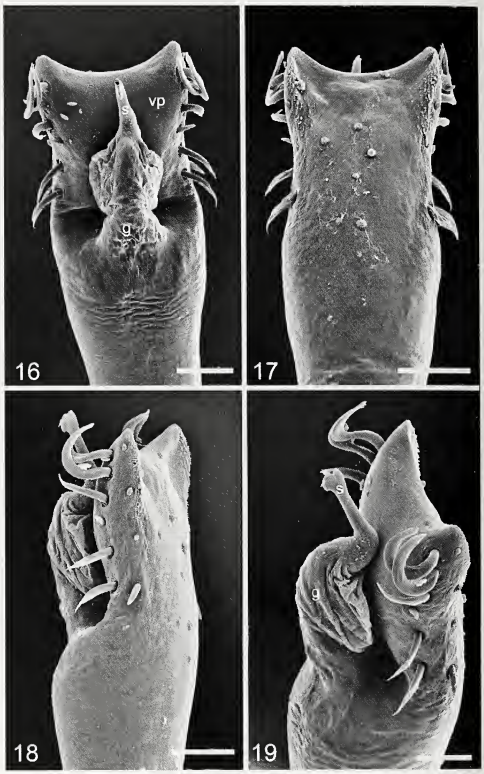
Remarks.—This species was previously known only from three specimens (female holotype, two male paratypes) collected at Barro Colorado Island, Canal Zone, Panama (Goodnight & Goodnight 1942, Goodnight & Goodnight 1947).

Bugabitia Roewer 1915

Bugabitia Roewer 1915:109; Roewer 1923:518; Mello-Leitão 1926:357; Roewer 1931:107; Mello-Leitão 1932:404; Soares & Soares 1949:231; Kury 1997:4; Kury 2003:207.

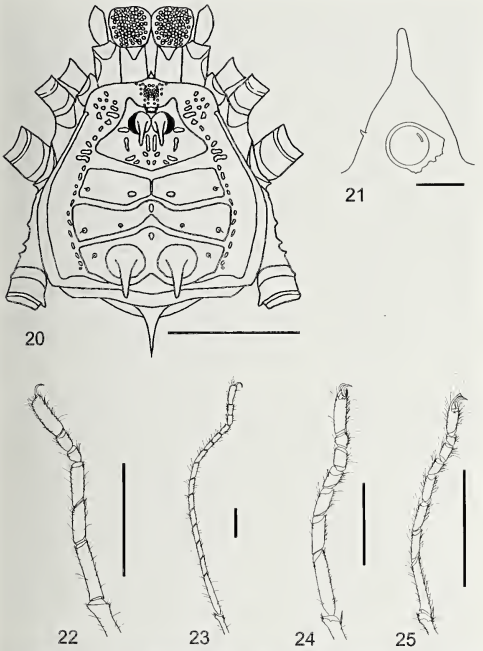
Type species.—*Bugabitia triacantha* Roewer 1915, by original designation.

Emended diagnosis.—Anterior margin of carapace with or without median spiniform tubercle. Ocularium with 2 or more tubercles each side, anterior granular tubercle smaller or with



Figures 16–19.—*Barrona williamsi* Goodnight & Goodnight 1942, penis, SEM: 16. Dorsal view of the distal portion of the penis; 17. Lateral view of distal portion of the penis; 18. Ventral view of the distal portion of the penis; 19 Lateral view of the distal tip of the stylus. Scale bars = 50 μm. Abbreviations: g = glans penis, s = stylus, vp = ventral plate.

3 small granular tubercles, similar in size. Abdominal scutum with 4 distinct areas; areas I and II unarmed or armed with paired granular tubercles; area III with paired spiniform tubercles that may or may not be encircled by a ring of smaller granular tubercles; area IV–V unarmed; anterior margin with a single median process. Lateral margins of scutum unarmed. First and third free tergites unarmed; second free tergite with a median spiniform tubercle. Anal operculum smooth. Pedipalpal femur and patella unarmed; tibia with 4 ectal (IiIi) and 5 mesal (IiiIi) spines; tarsus with 4 ectal (IiIi) and 4 mesal (IiIi) spines. Coxa IV with 5 or more small tubercles, similar in size; femora III–IV with paired, dorsal apical granular tubercles; tarsal formula: 6:14–16:7:8; tarsal claws unpectinate. Color of scutum dark brown, with yellow legs mottled with black; spiniform tubercles on abdominal scutal area III and second free tergite yellow or white, contrasting strongly with dorsum. Metatarsus I with distal expansion near joint with tarsus.



Figures 20–25.—*Bugabitia akini*, new species, female, holotype: 20. Habitus, dorsal view; 21. Ocularium, lateral view; 22. Tarsus I, lateral view; 23. Tarsus II, lateral view; 24. Tarsus III, lateral view; 25. Tarsus IV, lateral view. Scale bars = 2 mm (Fig. 20); 0.2 mm (Fig. 21); 1 mm (Figs. 22–25).

Basitarsus I of male spindle-like; basal 3 segments swollen. Male genitalia unknown.

Bugabitia akini, new species
(Figs. 1, 20–25)

Material examined.—PANAMA: *Coclé Province*: Holotype female, Parque Nacional General Division Omar Torrijos H., El Cope (08°49.2′80″N, 80°05′45.7″W), 23–28 February 2007, V. Townsend, A. Savitzky and J. Ray, collected by hand along hiking trails at night in montane rainforest (AMNH). Paratype: 1 female, collected with holotype (AMNH).

Etymology.—This species is a patronym in honor of Jonathan Akin who has made many contributions to the study of natural history and for his invaluable assistance on prior field trips.

Diagnosis.—Dorsal scutum pyriform with scutal areas poorly defined, areas I–II unarmed, area III with 1 spiniform tubercle each side not encircled by ring of smaller tubercles, areas IV–V indistinct and unarmed (Fig. 20). Ocularium with a spiniform tubercle and a smaller anterior granular tubercle each side (Fig. 21). Anterior margin of carapace unarmed (Fig. 20). Cheliceral sockets of carapace very shallow (Fig. 20). Cheliceral bulla smooth. Basal tarsal segments I of male swollen and spindle-like. Free tergites I and III unarmed; II with 1 median spiniform tubercle (Fig. 20). Femur and tibia IV straight. Tarsal formula 6:14–16:7:8. Tarsal claws III–IV unpectinate (Figs. 22–25). Penis: unknown.

Description.—*Female:* Measurements (holotype, in mm): dorsal scute length 3.53; cephalothorax length 1.32; mesotergum width 3.59; cephalothorax width 2.36; leg segments (length): trochanter I: 0.50; femur I: 4.43; patella I: 0.89; tibia I: 2.84; metatarsus I: 5.30; tarsus I: 2.00; total leg I: 15.96; trochanter II: 0.72; femur II: 11.41; patella II: 1.17; tibia II: 8.81; metatarsus II: 10.67; tarsus II: 5.67; total leg II: 38.45; trochanter III: 0.80; femur III: 7.59; patella III: 1.38; tibia III: 3.69; metatarsus III: 6.98; tarsus III: 3.34; total leg III: 23.78; trochanter IV: 0.90; femur IV: 10.36; patella IV: 1.59; tibia IV: 5.16; metatarsus IV: 9.99; tarsus IV: 4.23; total leg IV: 32.23.

Dorsum (Fig. 20): anterior margin of carapace with median spiniform tubercle; eye mound with a larger spiniform tubercle and a smaller, anterior granular tubercle each side (Fig. 21); abdominal scutum with 4 distinct areas; area I smooth with a few sparse spines; area II smooth with a few sparse spines; area III with paired spiniform tubercles not encircled by smaller tubercles at the base; areas IV–V smooth; lateral margins of abdominal scutum without tubercles. Free tergite I smooth; II with a median spiniform tubercle; III smooth. Anal operculum smooth.

Venter: coxae I–III with rows of granular tubercles bearing spines, IV with scattered granular tubercles bearing spines.

Chelicera: smooth with many setae.

Pedipalp: trochanter length: 0.37 mm; femur length: 1.55 mm; patella length: 0.70 mm; tibia length: 0.96 mm; tarsus length: 1.03 mm; total length: 4.61 mm; coxa, trochanter, femur, and patella smooth; tibia ectal IiIi, mesal IiiIi; tarsus ectal IiIi, mesal IiIi.

Legs (Figs. 22–25): coxa IV with 5 granular tubercles bearing spines; trochanters with few, small granular tubercles bearing spines; femora I–II smooth, femora III–IV with a pair of dorsal, apical granular tubercles; patellae-tarsi I–IV smooth with sparse spines; tarsal formula: 6:14–16:7:8.

Color: dorsum dark brown, with lighter, yellowish margins on abdominal scutum and free tergites; ocularium, paired tubercles on area III, and single tubercle on free tergite II yellow, contrasting strongly with dorsum; legs, chelicerae and pedipalps yellow mottled with black.

Male: Unknown.

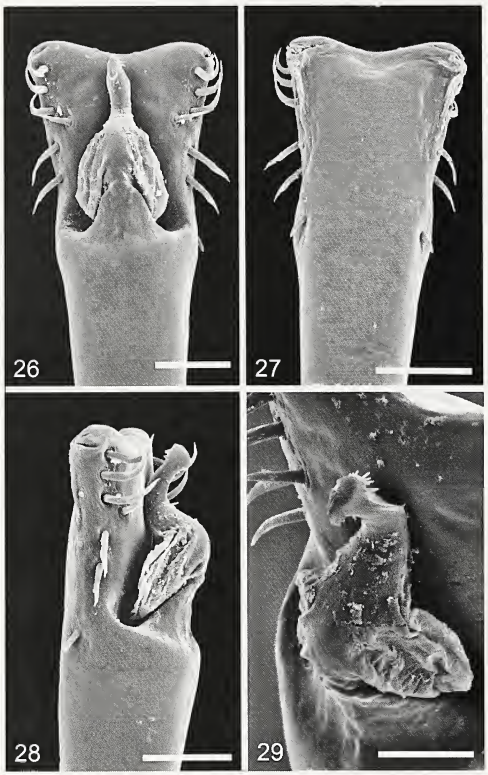
Habitat.—Same as *Barrona felgenhaueri*.

Remarks.—The holotype and paratype differ slightly in size and with respect to the morphology of metatarsus I. In the holotype, the distal region of this leg segment is noticeably expanded in comparison with that of the paratype. This morphology resembles that of the male holotype of *B. triacantha*, which also has a spindled basitarsus. The basitarsus of the female holotype of *B. akini* is not expanded.

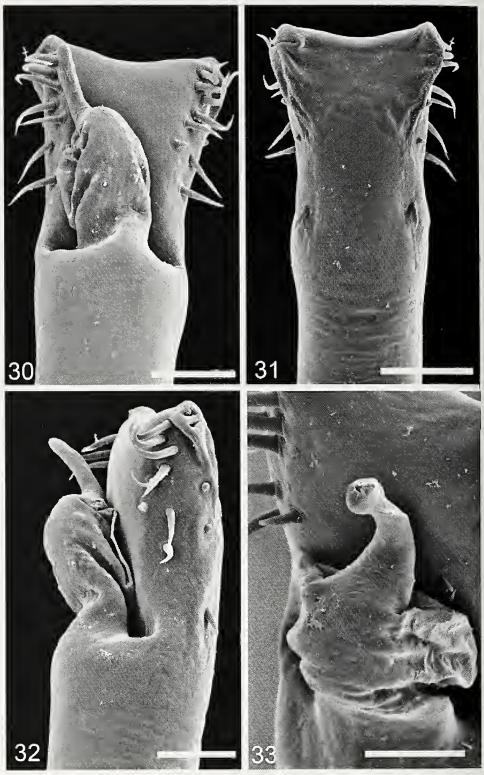
Cranellus montgomeryi Goodnight & Goodnight 1947a
(Figs. 26–29)

Cranellus montgomeryi Goodnight & Goodnight 1947a:6, figs. 11, 12; Kury 2003:207; Townsend et al. 2008a:59–60, figs. 2h, j; Townsend et al. 2008b:1027.

Material examined.—TRINIDAD, W.I.: 6 males, 6 females, Lalaja Trace (10°44′28.3″N, 61°16′17.3″W), July 2007, D.



Figures 26–29.—*Cranellus montgomeryi* Roewer 1932, penis, SEM: 26. Dorsal view of the distal portion of the penis; 27. Lateral view of distal portion of the penis; 28. Ventral view of the distal portion of the penis; 29. Lateral view of the distal tip of the stylus. Scale bars = 50 μm (Figs. 26–28); 25 μm (Fig. 29).



Figures 30–33.—*Rhopalocranaus albilineatus* Goodnight & Goodnight 1947, penis, SEM: 30. Dorsal view of the distal portion of the penis; 31. Lateral view of distal portion of the penis; 32. Ventral view of the distal portion of the penis; 33. Lateral view of the distal tip of the stylus. Scale bars = 50 μm (Figs. 30–32); 25 μm (Fig. 33).

Proud, captured by hand during the day in leaf litter along hiking trails in elfin woodland (AMNH); 3 males, 5 females, Morne Bleu Ridge, Northern Range (10°43′52.5″N, 61°15.7′0.7″W), July 2006, D. Proud and P. Resslar, captured by hand in leaf litter along hiking trail in montane rainforest (AMNH).

Description.—*Male genitalia* (Figs. 26–29)*:* Truncus long and slender; ventral plate defined as an elongate rectangle, tapering towards distal margin, with a distal border entire, slightly concave (Figs. 26, 27); lateral borders with 3 straight + 3 recurved setae (Fig. 28). Stylus straight with apex folded and unarmed (Fig. 29).

Rhopalocranaus albilineatus Roewer 1932
(Figs. 30–33)

Rhopalocranaus albilineatus Roewer 1932:285, fig. 3; Goodnight & Goodnight 1947a:8; González-Sponga 1991:205, figs. 29–36; Burns et al. 2007:140; Townsend et al. 2008a:59–60, figs. 2f, g; Townsend et al. 2008b:1027–1029, figs. 1e, f; Giribet et al. 2009:18.

Material examined.—TRINIDAD, W.I.: 10 males, 10 females, Mt. Tamana, Central Range (10°28′15.5″N, 61°11′50.5″W), July 2008, M. Moore and J. Toraya, captured by hand late in the afternoon in leaf litter from tropical seasonal forest (AMNH).

Description.—*Male genitalia:* Truncus long and slender; ventral plate defined as an elongate rectangle, tapering towards distal margin, with a distal border entire, slightly concave (Figs. 30, 31); lateral borders with 4 straight + 3 recurved setae (Fig. 32). Stylus straight with apex folded and unarmed (Fig. 33).

Remarks.—This species is very common in the leaf litter in most forested habitats island-wide. Individuals have been captured from leaf litter, tree buttresses and from beneath logs and rocks.

Table 1.—Interspecific variation in penis morphology among Manaosbiidae. Data for the South American species are based upon examinations of published figures, micrographs or descriptions (Šilhavý 1979, Kury 1997, 2007).

Species	Shape of the ventral plate	Shape of the distal border of the ventral plate	Setae on lateral border of ventral plate	Shape of the apex of stylus
Barrona felgenhaueri	Elongate, rectangular	Slightly concave	5 straight + 2 recurved	Folded
Barrona williamsi	Elongate, rectangular	Slightly concave	4 straight + 3 recurved	Folded
Cranellus montgomeryi	Elongate, rectangular	Slightly concave	3 straight + 3 recurved	Folded
"Isocranaus" strinatii	Elongate, rectangular	Substraight	6 straight	Folded
Manaosbia scopulata	Very elongate, rectangular	Parabolic cleft	4 straight + 3 recurved	Folded
Rhopalocranaus albilineatus	Elongate, rectangular	Slightly concave	4 straight + 3 recurved	Folded
Rhopalocranaus bordoni	Elongate, rectangular	Slightly concave	4 straight + 3 recurved	Folded
Saramacia alvarengai	Elongate, rectangular	Parabolic cleft	9 straight	Folded
Saramacia annulata	Elongate, rectangular	Parabolic cleft	8 straight	Folded
Saramacia lucasae	Elongate, rectangular	Parabolic cleft	8 straight	Folded
Syncranaus cribrum	Elongate, rectangular	Substraight	4 straight + 3 recurved	Papillate

Natural history.—Little is known about the natural history of harvestmen from the family Manaosbiidae. In Trinidad, Townsend et al. (2008a) reported that *Rhopalocranaus albilineatus* is a habitat generalist and exhibits an island-wide distribution. In contrast, *Cranellus montgomeryi* is a habitat specialist, with a distribution limited to montane rainforest and elfin woodland in the Northern Range. In montane rainforest, *R. albilineatus* was present, but not as common as *C. montgomeryi*. In Panama, only the natural history of *Zygopachylus albomarginis* has been examined (Rodríquez & Guerrero 1976; Mora 1990). During the course of this study, we had opportunities to observe manaosbiids from two sites: Parque Summit, a lowland seasonal forest near the Canal Zone; and Parque General Division Omar Torrijos, a montane rainforest near El Cope. At Parque Summit, *Barrona williamsi* is syntopic with *Z. albomarginis*. Individuals of both sexes from each species were observed occupying refugia beneath logs during the day. During a brief one-day survey, two adult male *Z. albomarginis* were observed residing within mud nests, but no eggs, nymphs or females were observed in these arenas. Male *B. williamsi* were found nearby, beneath adjacent logs or in spaces between the bark and wood of fallen trees. At Parque General Division Omar Torrijos, sampling occurred over a period of several days, mostly at night between 2000–2400 h. Individuals of four species, including *Barrona felgenhaueri*, *B. williamsi*, *Bugabitia akini*, and an undescribed species of *Zygopachylus* were collected from the litter and from beneath logs and small rocks. No individuals were observed occupying mud nests; however, a male-female pair of *B. felgenhaueri* was collected from beneath the same log. We did not observe any instances of feeding, reproductive behavior, or ectoparasites for the manaosbiids at Parque GD Omar Torrijos. All four species were found in the same microhabitat along either walking trails or forest edges.

Genital morphology.—With respect to penis morphology, harvestmen of the family Manaosbiidae possess a moderately long truncus, which is distally divided into a rectangular ventral plate and a dorsal distal-oriented glans that lacks dorsal or ventral processes and terminates in a stylus with a folded or papillate apex (Table 1). In this study, we described the penis morphology of *Barrona felgenhaueri* and *B. williamsi* from Central America and *Cranellus montgomeryi* and *Rhopalocranaus albilineatus* from the Caribbean and compared our observations with published descriptions of genital morphology for seven species from South America (Šilhavý 1979; Kury 1997, 2007). With respect to overall appearance, the penis morphology exhibited by *Barrona* spp. was most similar to that of *Rhopalocranaus* spp. and *Cranellus montgomeryi*.

However, we observed relatively little intrageneric variation (Table 1) in penis morphology. The only features that varied within the genera *Barrona*, *Rhopalocranaus* and *Saramacia* were the number and shape of setae on the lateral border of the ventral plate. Other characters associated with the penis including the shape of the ventral plate, the shape of the distal border of the ventral plate, and the shape of the apex of the stylus were conservative within a genus, but varied among the genera that we compared (Table 1). Most taxa possess an elongate, rectangular ventral plate, with the exception of *Manaosbia scopulata*, which has a very elongate ventral plate (Kury 2007). Most species also have a stylus with a folded apex, with the exception of *Syncranaus cribrum*, which has a papillate stylar tip (Kury 1997). The penises of *Manaosbia scopulata* and *Saramacia* spp. have a parabolic cleft in the distal margin of the ventral plate, in contrast to other taxa, in which the margin may be slightly concave or even substraight (Table 1). With respect to other families of harvestmen within the Laniatores, variation in penis morphology within the Manaosbiidae appears to be relatively conservative, similar to levels reported for the Cosmetidae (Kury et al. 2007; Townsend et al. 2010), and considerably less diverse than that observed for the Gonyleptidae (Kury & Pinto-da-Rocha 2007) or Oncopodidae (Schwendinger & Martens 2002).

The functional significance of genital morphology has received relatively little attention within the Gonyleptoidea or the suborder Laniatores. Currently, the functional aspects of genital morphology have only been explored in the Oncopodidae (Schwendinger & Martens 2002).

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The hub as a launching platform: rapid movements of the spider *Leucauge mariana* (Araneae: Tetragnathidae) as it turns to attack prey

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Abstract. Spiders are effectively blind with respect to the lines in their webs, and they commonly use exploratory leg movements to find lines, just as a blind man finds objects using a cane. Nevertheless, a mature female *Leucauge mariana* (Keyserling 1881), which spins a relatively open, sparsely-meshed hub and whose legs I and II hold widely-spaced radii rather than dense hub lines, turns precisely and rapidly when prey strike her orb. She can turn > 90°, finding and grasping new lines with all her legs, in as little as 0.1 s and can reach a prey several body lengths away in as little as 0.23 s after impact. The hub design and resting postures of the spider's legs allow her to sense where the prey strikes the web, generate the force necessary to turn her body rapidly, and find lines to grasp. The spider may move most (if not all) of her legs, without obtaining further guidance information once the leg has begun to move until it nears the site where it will grasp a line. The order in which legs are moved is relatively consistent, and each tarsus moves to a site where lines are relatively abundant; some then make small, quick searching movements to find and grasp lines there. When radial lines were experimentally cut near the hub in a sector in which a prey was subsequently introduced, legs I and II first made small searching movements, and then executed much larger searching movements. The rapid leg movements directed toward specific areas where lines are abundant, and the small searching movements employed at these sites suggest that the spider modifies her behavior when she is at the hub of an orb.

Keywords: Leg movements, rapid orientation behavior, orb web design

To move in an orb web, a spider must first find lines before it can grasp them. Orb weavers are likely to be unable to see the lines in their webs and are thus essentially blind with respect to the positions of these lines. This is because many species build and operate their webs in the darkness, and the eyes of orb weavers are incapable of resolving such fine lines (Foelix 1996). In addition, their eyes are placed dorsally, while the lines are generally ventral to the spider's body. In most contexts, the spider's solution is to use its legs as tactile sense organs, waving and tapping with them like a blind man using his cane (e.g., Hingston 1920, 1922; Witt et al. 1968; Eberhard 1972; Vollrath 1992). An orb weaver's task is more difficult than that of a blind man, however: it has eight different legs, and it needs to find highly localized supports (the lines in its web) to sustain its weight.

Despite these problems, orb weavers generally take only a few seconds to reach insects that strike their webs. Average response times, from the moment of prey impact until initiation of biting or wrapping, were 6.9 s in *Nephila maculata* (Fabricius 1793) and 8.7 s in *Cyrtophora moluccensis* (Doleschall 1857) responding to blowflies (Lubin 1973), about 5.5 s in *Araneus diadematus* (Clerck 1757) responding to house flies (Witt et al. 1978), and from 1.7 to 3.8 s in *Cyclosa turbinata* (Walckenaer 1842) (R. Suter pers. comm.). Execution of such rapid responses to prey is physically challenging. By following the movements and positions of a spider's legs as they touch or grasp lines, it is possible to deduce the information it has available regarding the positions of lines, just as one can deduce from the movements of a blind man's cane which objects he has succeeded in locating as he moves through the environment.

One common tactic that spiders use to locate lines is following behavior (Eberhard 1972). First a more anterior leg explores the space in front of the spider's body by waving and tapping, and finds and grasps a line there. Then the spider moves a more posterior leg forward and grasps the same line near the site held by the anterior leg. Then the anterior leg moves forward to explore for further lines. In this way a line is passed from one leg to the next and so on, and more posterior legs do not need to search for lines. Following behavior is probably widespread. It has been seen in a nephilid (Hingston 1922), a uloborid (Eberhard 1972), a tetragnathid (Eberhard 1987a), and several araneids (Jacobi-Kleemann 1953; Eberhard 1982; W. Eberhard unpubl. data on *Micrathena duodecimspinosa*) (Cambridge 1890).

Following behavior, however, is probably too slow for a spider at the hub of its orb when a prey strikes the web. Prey often escape quickly from orbs, and in many orb weavers more than half of the prey that strike the web escape (summary in Eberhard 1990), so the spider needs to turn rapidly toward the prey. Indeed, some spiders do respond quickly and precisely; the beginning of the response of *Nephila clavipes* (Linnaeus 1797) to vibrations occurred after a delay of only 0.1 s, and the spider turned to face the prey (with a precision of 3.6 ± 7.7°) (mean ± standard deviation) in only 0.04 s (Klärner & Barth 1982); corresponding times for *Zygiella x-notata* (Clerck 1757) were 0.1 and 0.6 s (Klärner & Barth 1982).

How are spiders able to accomplish such rapid reactions without being able to see the lines on which they depend for support? In some orb weavers, such as *Cyclosa turbinata* and *N. clavipes* (Suter 1978; Klärner & Barth 1982), the mesh of the hub is very tight, so lines are available nearby for all of the spider's tarsi to grasp wherever they are placed. In other species, however, such as many tetragnathids, the center of the hub is open (perhaps an adaptation to increase the web's ability to sag when prey strike it – Eberhard 1987a), and the

hub itself has relatively few lines, so more precise placement of the tarsi is necessary. In this study, we used high speed video recordings and experimental manipulations of webs to address the question of how *Leucauge mariana* (Taczanowski 1881), a species with an open, loosely meshed hub, executes attacks even more rapid than those measured in other species.

METHODS

We used mature females of *L. mariana* for all observations and recorded behavior in captivity using a high-speed video camera (up to 500 frames/s) (TroubleShooter® model TS500MS Fastec Imaging Corporation - www.fastecimaging.com) connected to a computer. The camera recorded continuously, maintaining a record (buffer) of the latest 2 s in the computer's memory. By stopping the camera within 2 s after an event had occurred, we saved the recording of the event in the computer's memory.

We collected intact webs of mature females in San Pedro de Montes de Oca, Costa Rica. After removing the spider from her web and placing her in a vial, we pressed a circular styrofoam frame coated with double-sided sticky tape carefully against the anchor lines of the more or less horizontal orb; then we cut these lines free from the objects to which they were attached. We took care to minimize alterations in the tensions on the web, and if the tensions in a web seemed to have been altered, we discarded the web in favor of another. We reintroduced the spider onto her web after placing it horizontally over a strong (1000 W) light and a black background. We directed the camera downward from above, and focused on the hub of the web; all or most of the radii and hub lines were visible in the recordings.

We assigned females randomly to one of three treatments. For females in the "3 radii cut" experiment, we gently cut three adjacent radii in a sector behind the spider (between 90° and 180° from the direction in which she was oriented) in the free zone (the space lacking spirals between the hub and the inner loop of sticky spiral) with scissors while the spider rested at the hub (Fig. 1a). This manipulation (to which the spider usually gave no overt response) produced a hole in the array of radii near the hub. Given that orbs of this species have on average about 30 radii (Eberhard 1988), interradial angles averaged approximately 12°, and the hole in an orb with three adjacent radii broken was on the order of 48°. For experimental females in the "all but 5 radii cut" treatment, we cut all but five radii in the free zone, leaving five intact radii at approximately equal angles (Fig. 1b). The mean angle between adjacent intact radii was thus on the order of 72°. The orbs of control females were left unaltered.

We elicited turning reactions of spiders by gently blowing live *Drosophila melanogaster* flies from an aspirator held perpendicular to the web. The fly struck a portion of the web to the rear of the spider, between 90° and 180° from the direction in which she was oriented, and approximately half way from the hub to the frame. The fly was not always in the field of view in the recordings, but in some recordings the vibration caused by its impact was visible, and the lapse between impact and the first response of the spider could be determined.

Leg movements were presumed to function as exploration when the tarsus moved in a tapping or waving pattern until it contacted a line, and then immediately seized and held this line (Fig. 2). Similar movements that did not result in contact with lines were also considered to be exploratory. Legs on the side of the spider toward which she turned are termed leading (or L) legs, while those on the other side are trailing (or T) legs. Means are followed by ± 1 standard deviation.

We also studied the behavior of mature females in the field in San Pedro de Montes de Oca, and near San Antonio de Escazu, Costa Rica. We recorded the resting postures of the legs of spiders in the field in two ways. We noted which radii held by legs I and II by direct observations. In addition, we used digital photos of spiders as they rested at the hub to measure the angles between adjacent legs using the program "Image J" (Image J. 2006. Image J. http://www.uhnresearch.ca/facilities/wcif/imagej/, Bethesda, Maryland, USA) (Fig. 3). We studied responses to prey by dropping a 2.75 mg weight (a V-shaped 1.1 cm piece of fine copper wire) onto the outer half of the sticky spiral portion of the web to the rear of the spider (90° to 180° with respect to the orientation of her body) from about 1–2 cm above the web. Mature female *L. mariana* weigh approximately 40–60 mg (Eberhard 2007), so these weights were on the order of 5% of the spider's body weight. We filmed the responses of spiders at 30 fps with a digital movie camera (Sony DCR-TRV50). Because the radii were more reliably discerned with the naked eye, we also observed the orientation of other spiders directly. We only used spiders that were on intact orbs and that were not feeding. No spider was observed more than once.

RESULTS

Resting leg positions in the field and distribution of weight.— To aid in understanding the details of turning behavior, we first describe the spider's original position while resting at the hub. This position was relatively consistent (Table 2, Fig. 3, 0:012 in Fig. 4). Legs I and II always held radii beyond the edge of the hub, nearly always in the free zone (rarely extending into the prey capture zone), while legs III and IV usually held either radial lines or hub loops within the hub (Table 2). Legs III were directed laterally; the angle of the tarsi with the central axis of the spider averaged 89.5 ± 9.1° (range 72–111°). The positions of the two legs III tended to be bilaterally symmetrical, as there was a significant positive correlation between the angle of one leg III and that of the other ($R = 0.45$, $P = 0.014$). Legs IV gripped the web in approximately symmetrical positions directed posteriorly (Fig. 3). The separation between legs I was greater than that between ipsilateral legs I and II, both in terms of the angles between legs, and in terms of unoccupied radii between them (Table 2). The tip of the spider's abdomen was always in the hole in the center of the hub (Table 2), often near the center of this hole (Fig. 3).

There were three indications that legs IV, and probably also legs III, were more important in sustaining the spider's weight than legs I and II. First, the webs of *L. mariana* generally slanted somewhat with respect to horizontal (mean = 40 ± 13° in 66 orbs in the field – Eberhard 1987b), and undisturbed spiders on slanting webs nearly always faced downward. Thus legs IV were directed more nearly upward; their tarsi were above the others and thus probably sustained a greater portion of the spider's weight. Secondly, tarsi III and IV often

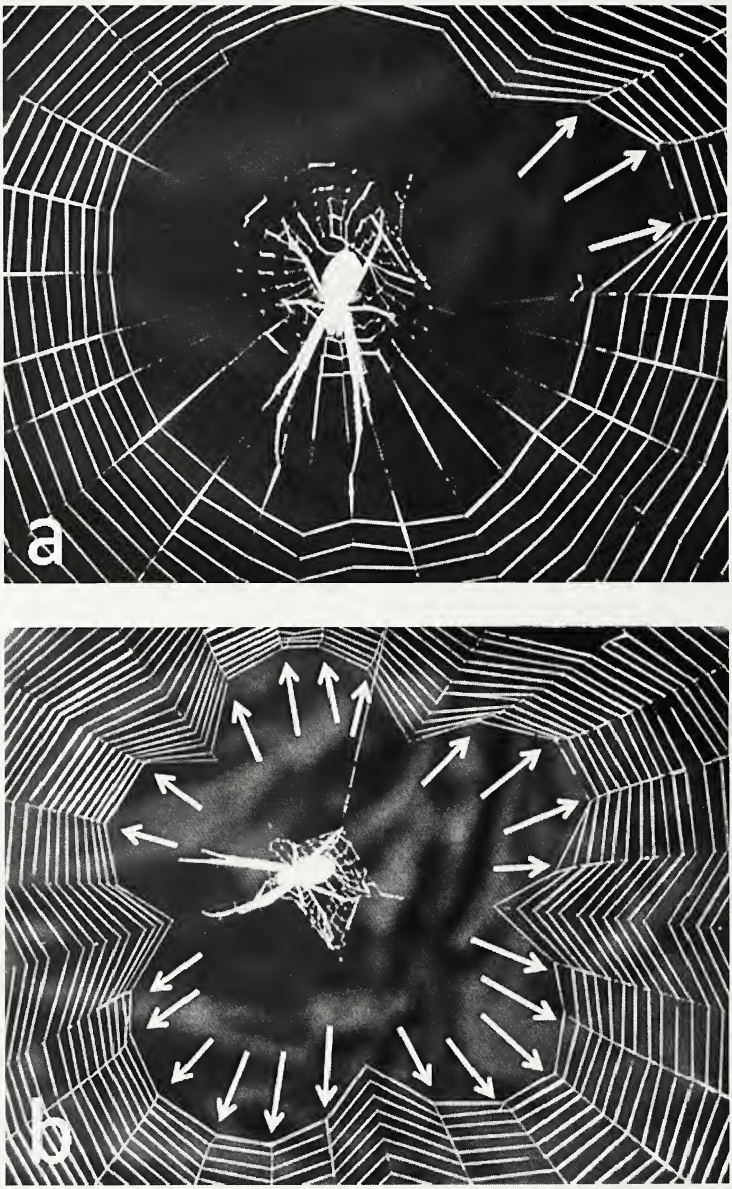

Figure 1.—Spiders resting at the hub of webs in which three radii were cut in the free zone in an area behind the spider (a), and in which all but five more or less equally spaced radii were cut in the free zone (b). Arrows indicate broken inner ends of radii (not all intact radii are clearly visible near the hub). Left legs I and II of the spider in b were held in the open space where radii had been broken, while right legs I and II held the same intact radius.

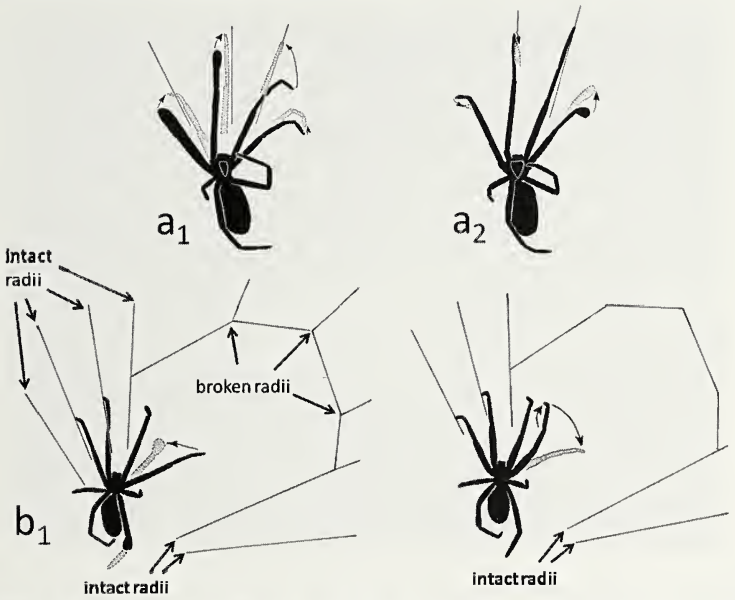

Figure 2.—Examples of movements in a small amplitude, rapid "J" (curved, thin arrows in a) and a slower, large amplitude (curved, thin arrows in b) exploratory movement in the 3 radii cut experiment. The solid image in a_2 occurred 0.002 s after the stippled image in a_1, while the stippled images in both a_1 and in a_2 were 0.006 s after their respective solid images; the solid image in b_2 occurred 0.064 s after the stippled image in b_1, while the solid and stippled images in b_1 and b_2 were 0.064 s and 0.144 s after their respective solid images.

pulled the lines they held into perceptible V configurations (e.g., leg TIV in frame 44 in Fig. 4), while such visible deflections of lines were rare for other tarsi. Finally, the abdomen constituted a mean of 71% of the total fresh weight of three individuals (none were obviously swollen with eggs; mean weight 36.6 mg), while the legs constituted only about 17% and the cephalothorax 12% of her weight (the percentage in the abdomen will obviously be greater in females about to oviposit). Therefore, the center of gravity of a mature female probably lies somewhere in the anterior portion of her abdomen. Usually the only legs posterior to this were legs IV; legs III were approximately lateral to the abdomen-cephalothorax junction, and thus probably somewhat anterior to the spider's center of gravity.

When the spider was at the hub, she was apparently able to distinguish intact from broken radii, perhaps on the basis of the resistance they offered when she pulled on them. When the spider was chased to the edge of the web and alternate radii were cut beyond the free zone but near the inner edge of the prey capture zone (all radii were cut less than seven loops of the sticky spiral from the innermost sticky spiral loop) in the lower portion of the web (where her legs I and II would be), legs I grasped unbroken radii in 71% of 154 radii in 77 webs, and legs II grasped unbroken radii in 67% (both significant: $P < 0.001$ with X^2 tests) when the spider returned to the hub and resumed her resting posture. Results from a second experiment in which we cut additional radii suggest that this preference for intact radii may be due to a preference for radii that give less when the spider pulls on them. When we cut alternate radii farther from the free zone (near the frame) in 51 additional orbs, the preference for intact radii was reduced. Because orbs typically have approximately 40 loops of sticky spiral (Eberhard 1988), these radii had approximately 30 loops of sticky spiral attached to the inner intact segment of the radius that was nearest the hub. The preference of legs I for intact radii disappeared (50% of legs I were on unbroken radii), while the preference of legs II for intact radii remained, but was slightly weakened (63% on unbroken radii).

Speed of response.—Each spider performed three basic tasks as she turned at the hub in response to prey: locate and grasp the radial lines leading toward the prey with her anterior legs, pull and push on lines at the hub so as to turn her body until it faced toward the prey, and reposition all her other legs in preparation to run toward the prey. Different functions were performed by different legs. As in other orb weavers (e.g., Suter 1978; Klärner & Barth 1982), attack behavior by *L. mariana* began with the spider turning rapidly at the hub to face the prey. The mean delay between the impact of the prey and the first movement of the spider's anterior legs in high-speed video recordings in control webs was 0.055 ± 0.04 s (minimum 0.012 s) (n = 14). These response delays (which somewhat underestimate the spider's speed, since they do not include the flexion of legs III and IV that just preceded the movements of legs I and II – see below) were comparable to

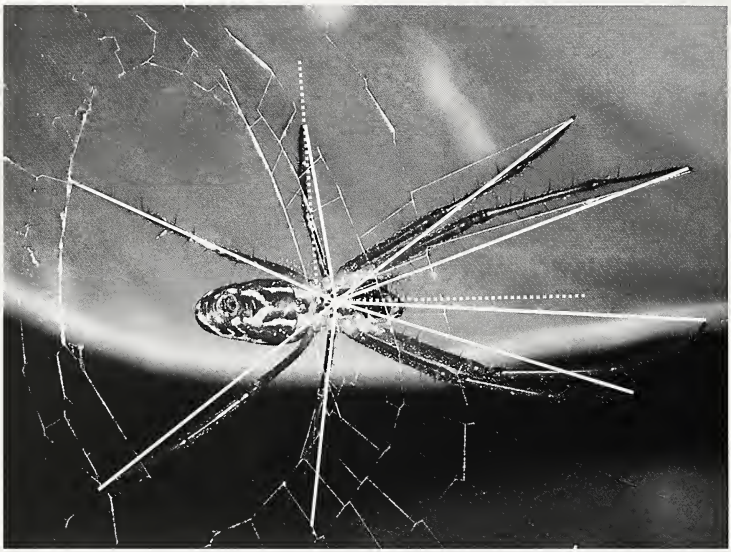

Figure 3.—An adult female *L. mariana* resting at the hub of her web. The solid lines mark the angles that were measured between her legs, and the dotted lines the angle between her longitudinal axis and one leg III.

delays seen in the field, which lasted a median of one frame in a video recording (0.03 s). Mean delays were similar in high-speed video recordings of "3 radii cut" webs (0.117 ± 0.101 s, minimum 0.028 s) (n = 25); but the delays were longer in "all but 5 radii cut" webs than in control webs (0.177 ± 0.115 s (minimum 0.03 s) (n = 26) ($P < 0.001$ with Mann-Whitney U Test).

In the 20 cases recorded in the field in which the spider ran to the wire, she took as little as four more frames (about 0.13 s) to move 4–5 body lengths and touch the prey with her anterior legs. Thus the shortest total delay in the field, from the impact of the wire until the spider touched the wire with her legs I, was 7 frames (about 0.23 s) (two cases) (two other spiders took only 0.33 s). Not all delays were this short, and the median was 16 frames (0.53 s). Commonly, the spider jerked the web at the hub one or more times after turning and before running toward the prey when the delay was longer. Once the spider began to run toward the prey, her mean velocity was 29.6 ± 7.7 body lengths/s (n =12; the mean distance travelled in these cases was 6.5 body lengths; body length in this species is on the order of 7 mm).

Leg movements during turning behavior on control orbs.—Several details of how the spider turned to face the prey were relatively consistent in high-speed video recordings.

Early movements: The first movements were small flexing movements of legs LIII and LIV that drew the web lines held by their tarsi (and connected lines) toward the spider's body. These just barely visible tensing movements were simultaneous, and generally preceded the first lateral movement of other legs by 0.002–0.004 s (1–2 frames of high-speed video). These tensing movements presumably helped generate the force needed to swing the spider's legs and body laterally and rearward (note TIV in Fig. 4, 0:044). Leg LIII continued to pull on the web (and thus probably produced a turning force) until it released its hold on the hub (and the hub lines that it had pulled on sprang back to their previous positions). Leg LIV maintained its hold much longer; it ended up being bent far under the spider's body (Fig. 4, 0:080) before finally releasing its hold.

Legs LI and LII were usually the first to move laterally, releasing the radii they were holding, descending somewhat below the plane of the web, and swinging simultaneously laterally and rearward toward the side of the hub where the prey had landed (0.044–0:060 in Fig. 4). LII usually began to move either simultaneously or only about 0.002 s later than LI (Table 2, Fig. 5), and the two legs swung almost as a unit, with their tips remaining nearly the same distance apart during the entire lateral and rearward swing (Figs. 4, 0:044, 0:060). After reaching an orientation more or less toward the prey, the two legs moved upward and grasped new radial lines, about 0.05 s after they had begun to move (Fig. 5). Neither leg made any perceptible tapping or waving movement during the swing, and neither leg consistently ended up grasping a line that was held by any other leg; thus, the lateral swings of legs LI and LII were probably not guided by further stimuli from the web once they were initiated.

When legs LI and LII arrived in the sectors in which they would each grasp a radius, they each usually made a small, apparently exploratory movement (Fig. 2a). Usually tarsi LI and LII had not struck radii during the turn, and each was in a space between two radii; the leg was extended quickly upward and prolaterally and then flexed in a small "J" movement that

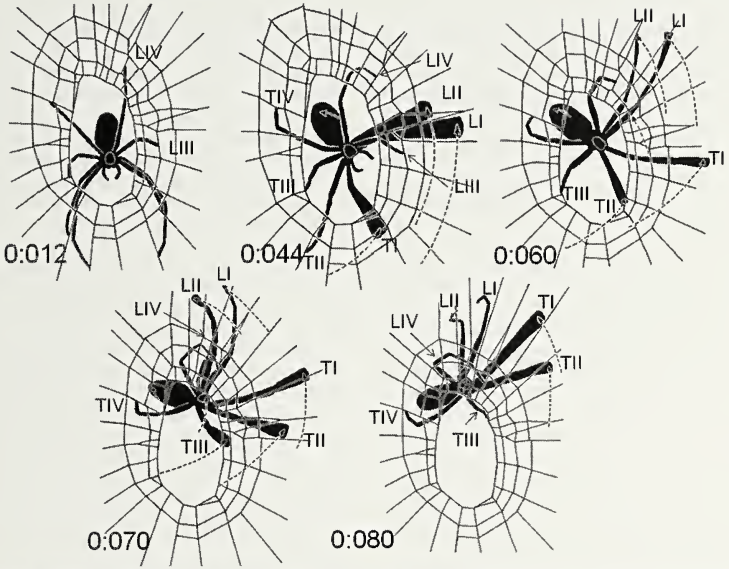

Figure 4.—A typical sequence of movements as a mature female *L. mariana* turned at the hub to face toward a *Drosophila* fly which had struck her web (traced from a view of her ventral surface from above in a high speed video). The times refer to fractions of a second elapsed following the frame of the video recording in which the first leg movement occurred. Thicker leg outlines indicate blurred images (i.e., structures moving rapidly); arrows with dotted lines represent distances that structures moved from preceding positions. Images of lines were generally not clear enough to be sure regarding deflections of lines due to tarsi pulling on them, and (other than TIV in "0:044") deflections are not included. Leg LIII was too indistinct in several frames to draw with certainty, and was omitted.

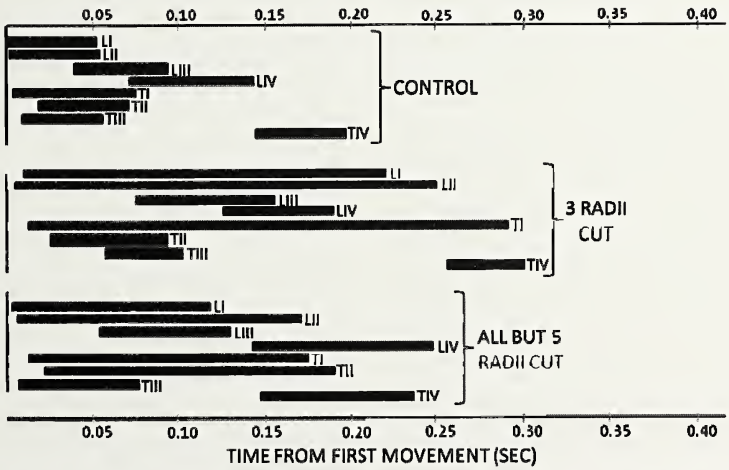

Figure 5.—Mean durations and sequences of leg movements during turns in the three treatments. Time 0 was the frame in the video in which the first leg movement occurred. The left end of each black bar represents the mean time at which the leg began to move, and the right end the mean time at which it grasped a new line. Sample sizes for the three treatments were, in order, 20, 20, and 25.

ended when the tarsus seized a radius. The leg always moved prolaterally (Fig. 2a), although the degree of extension varied. The presence and form of searching movements was flexible, and the order with which legs LI and LII seized radii apparently depended on the luck of where the tarsi arrived after the turn (how close they were to radii). "J" movements were often very small, and sometimes absent or so small as to be imperceptible. On average, LI grasped a new radius only about 0.002 s before LII (Fig. 5); sometimes leg LI was the first to grasp a line, sometimes LII, and sometimes they grasped radii simultaneously.

The radii seized by LI and LII were always adjacent to each other, for two reasons. First, the distance between the two legs was more or less constant as they were swung laterally and then began "J" movements and was similar to the distance between them before the turn began. Because the spider tended to face toward the larger part of the orb and her ipsilateral legs I and II generally held adjacent radii, only a single radius was present between them when they finished the first part of the turn. Secondly, the "J" movements of both legs were oriented prolaterally, thus eliminating the possibility that a third radius would remain undiscovered between them, which could have happened if leg II were to find a radius by moving retrolaterally while leg I found a radius by moving prolaterally. Thus, leg II always ended up seizing the radius that was between legs LI and LII after they had completed the turn. Within an average ca. 0.05 s after the turn began, LI and LII had grasped adjacent radii; usually the radius held by LI was the radius closest to the prey and would serve as the spider's attack route (below).

Soon after the anterior legs moved, leg TIII moved rapidly, crossing the hub hole to grasp a hub line near the opposite edge (Fig. 4, 0:060–0:070). This step by TIII was often quicker than that of any other leg (Fig. 5). Because legs III are relatively short, this step was necessary to allow the spider's body to turn. Associated with this movement, the tip of the spider's abdomen moved posteriorly (Fig. 4, 0:060, 0:070); subsequently, the point around which her body pivoted during the rest of the turning movement was near the tip of her abdomen (Fig. 4, 0:070–0:080).

Intermediate movements: Legs TI and TII trailed behind legs LI and LII in space and often in time. Sometimes TI began to move at the same moment when legs LI and LII moved, but more often it did not release its radius until a few hundredths of a second later (on average 0.02 s) (Fig. 5). The movement of TI began when it released the radius it was holding and swung downward and laterally across the spider's body toward the side with the prey (Fig. 4, 0:044). If its tarsus did not immediately encounter the radius adjacent to the radius held by LI, it searched with a prolateral "J" movement. In each of 30 cases the first line grasped by TI was the radius adjacent to the radius grasped by leg LI. In 27 of 30 cases TII then grasped the radius that was adjacent to the radius being held by TI.

Late movements: Legs III and especially legs IV were probably used to support the spider's body during the entire sequence. They held the web during the early stages of a turn without changing their grips as the spider's body turned and her more anterior legs were in the air moving rearward, and LIV, LIII and TIV only moved after the turn was nearly complete (Figs. 4, 5). The result was that Leg LIV became severely contorted and crossed over much of the spider's body (Fig. 4, 0:080). The twisted position of LIV suggested that the line gripped by its claws must have been severely twisted (perhaps twisted around the tip of the spider's leg), but there was never any sign that the spider experienced any difficulty in releasing the line held by leg LIV when she finally moved it. Leg TIV generally did not change its grip on the web until LIV had moved and seized another line (Fig. 5).

Once the spider had turned her body, she often jerked the radii one or more times with her anterior legs just before running toward the prey. The number of jerks in high-speed recordings ranged from one to three (mean = 1.42 ± 0.67 s, n = 15). The duration of a jerk averaged 0.04 ± 0.001 s, and the total time spent jerking averaged 0.076 ± 0.06 s) (n = 21). The most common combination of legs that jerked was both legs I and leg LII (Table 4).

Turning on experimental webs.—The responses of spiders on webs with radii that had been experimentally broken were similar in several respects to those of spiders on intact webs. The first tensing responses in the two types of experimental webs occurred 0.003 ± 0.002 s and 0.004 ± 0.002 s before the anterior legs began to move (not different from control webs). The spiders' body turned 158 ± 11°, 147 ± 20 °, and 151 ± 12 ° in, respectively, control, "3 cut radii", and all but "5 radii cut" treatments (again not statistically different). The order in which legs then initiated lateral movements was also similar to that in the controls (Fig. 5, Table 3). When legs LI and LII arrived in the area of the broken radii, however, their behavior differed. The original "J" movements failed to contact a radius, and at least one of the two legs then executed one or more large searching movements (Fig. 2b). Much more time elapsed before the legs finally grasped radii (Fig. 5, Table 2).

The spiders' jerking behavior also differed on experimental webs. The frequency with which the turn was followed by jerking behavior was not different from that in control webs (77% of 21 turns) in webs with three radii cut (76% of 70 turns) or with all but five radii cut (70% of 30 turns). However, the number of jerks following the turn increased, compared to the number observed on control webs (mean 1.42 ± 0.67): there were 2.17 ± 1.42 jerks in webs with 3 cut radii, and 2.83 ± 1.42 in webs with all but 5 radii cut (n = 70, 30; P = 0.02 and 0.004, respectively, compared with control values using Mann-Whitney U Tests). The mean duration of a jerk on experimental webs was not significantly different (0.046 ± 0.01 and 0.05 ± 0.013 s, respectively (n = 70, 30), compared with jerks on intact orbs (0.04 ± 0.001 s). Fewer legs were used to perform jerks on experimental webs than on eontrol webs (Table 4), presumably because fewer radii were available to be jerked.

Precision of turns in the field.—Spiders observed in the field generally responded immediately to the impact of "prey" (67% of 72 cases; presumably at least some failures to respond occurred because the spider had been inadvertently frightened by the observer contacting nearby vegetation). Of the 48 spiders that responded immediately, 89.6% turned accurately to face toward the prey, with one of the spider's legs I holding the radius running most directly toward the wire. In 79.2% of the immediate responses, the spider immediately ran to the wire (in the others she turned back to her resting position, possibly because the wire "prey" did not produce further

vibrations). In 71.4% of the 21 cases in which her orientation was correct and it was possible to see this detail, leg LI rather than TI held the radius nearest the wire ($X^2 = 3.86$, $df = 1$, $P < 0.05$). Thus the turn tended to undershoot rather than overshoot the correct radius. There was a similar trend in the mistaken orientations: in three of the four cases in which this detail was noted, the spider was short of the correct radius. Because the orbs of this species generally have on the order of 30 radii (Eberhard 1988), the precision of correct turns was on the order of ± 12° (the approximate angle between adjacent radii).

DISCUSSION

Speed of turns.—Compared with the webs of many orb weavers, those of *L. mariana* probably retain prey relatively briefly (Zschokke et al. 2006). Their orbs are relatively open-meshed, weak, and horizontal, and, compared with the spider's body size, have relatively small amounts of sticky material on sticky spiral lines (Opell 2002). Perhaps in association with their flimsy webs, the attack behavior of *L. mariana* is very rapid. The spider's reaction time – the time between prey impact and the first movement of her legs – was as little as 0.012 s, and averaged only 0.055 s in controls, or about half the 0.1 s reaction times of *Zygiella x-notata* and *Nephila clavipes* (Klärner & Barth 1982). The median of the total time to reach the prey in *L. mariana* (time between prey impact and the spider's legs contacting the prey) was only 0.53 s; the minimum was 0.21 s. These are substantially quicker responses than the mean of about 1.5 s reported for a combination of *L. mariana* and *L. venusta* (Walckenaer 1842) by Zschokke et al. (2006), perhaps because the prey in the present study were smaller (2.75 vs. mean of 14.4 mg in the Zschokke et al. study) and thus elicited less cautious approaches. The responses of other species of orb weavers are in general slower, with means ranging from 1.7 to 8.7 s (Lubin 1973; Witt et al. 1978; Zschokke et al. 2006; R. Suter pers. comm.). These comparisons underestimate the advantage in speed of *L. mariana*, because (in contrast with the other studies) all prey in this study hit the orb behind the spider and thus required a relatively large turn by the spider, probably slowing the speed of her attack.

Despite the speed with which *L. mariana* responded, the turn was also very accurate; in about 90% of turns of > 90°, the spider grasped the radius nearest the prey with one leg I. The angle she turned tended to be the minimum rather than the maximum needed (the leading leg I was more than twice as likely as the trailing leg I to grasp the correct radius), perhaps an additional feature designed to increase attack speed. In sum, we speculate that raw speed probably plays an important role in the predatory strategy of *L. mariana* (see also Zschokke et al. 2006). This gives reason to analyze the leg movements that were used to turn at the hub in terms of their effects on the speed of the spider's turn.

During the 0.1 s in which the spider turned on an intact orb, she found new lines to grasp with all eight legs. The largest leg movements appeared to be blind with respect to particular lines: the legs all seized lines that were not already being held by other legs, and no leg performed any exploratory behavior until it had arrived at the site where it would grasp a line. Once at these new sites, legs either grasped lines without any perceptible exploratory movements, or with only small "J" exploratory movements. The movements of both legs I, of both legs II, and of TIII were all initiated before any other legs had grasped a new line. If these movements of the spider's legs were not guided by further information once the leg began to move, as proposed here, they were probably guided on the basis of information obtained from the vibrations produced by the impact of the prey, conducted along the radii, and sensed by the spider's legs as they rested at the hub (Figs. 3, 4, 0:012). Probably the spider determined the direction of the prey by comparing the intensities of longitudinal vibrations of different lines (Landolfa & Barth 1996), and presumably the locations of prey that struck the web behind the spider were sensed mainly by her legs III and IV, on or near the radii closest to the prey. The probable importance of radii in transmitting vibrations is supported by the nearly threefold increase in the delay before the spider began to turn when all but five radii were cut (a mean of 0.18 s as opposed to 0.055 s, $P < 0.001$ with Mann-Whitney U Test), perhaps due to reduced amplitude of the vibrations or a greater difficulty in localizing their source.

The positions of the spider's legs at the hub surely influenced the leg movements needed to make a turn. The most interesting possible functional consequence was that the relative positions of LI and LII (Table 1) were maintained with little variation during the entire turn. Moving these legs as a unit may increase the likelihood of their grasping adjacent radii following the turn. This meant that if the spider's turn was slightly less than that needed to put her leg LI on the radius with the prey, her leg LII would occupy the radius on which the prey was located. The especially close space between legs I and II could also function to increase the speed with which the spider located the line leading to prey. Leg TI often trailed behind leg LI, but nevertheless consistently seized the radius adjacent to that seized by LI, however, so movement as a unit is not necessary to grasp adjacent radii.

All legs were moved during turns of > 90°, and in all cases their tarsi went directly to sites where lines were relatively closely spaced. Perhaps the most dramatic movement of this sort was that of TIII, which went directly from one edge of the hole in the center of the hub to the other (Fig. 4, 0:070). By moving her legs to sites where lines were abundant, the spider was able to find and grasp new lines with only small, quick searching "J" movements. We interpret these small "J" movements, which contrast with the large sweeping searching movements seen in other contexts, as being specially designed for web regions with abundant lines. The highly directed movements of legs to areas where lines were close together, and the use of "J" movements thus imply prior knowledge by the spider of the relative abundance of lines in different regions of the webs. The cue or cues that trigger such expectations remain to be established.

Precision of turns and motive force.—As just noted, the positions of the spider's legs as she rested at the hub probably influenced the information available from vibrations produced when the prey hit the web. Strikingly, however, the spider's legs were not positioned so as to obtain uniform coverage of vibrations from all parts of the orb. Instead, the angles between adjacent anterior legs (I and II) were much smaller than those between the posterior legs (III and IV), and the

Table 1.—Means ± standard deviations of angles and numbers of radii between adjacent legs and frequencies with which they grasped different sites for mature *L. mariana* females resting at the hubs of their orbs in the field. Values followed by the same letter and number were significantly different in Mann-Whitney *U* Tests ($P < 0.0001$).

Legs	Mean angle (°)	*n*	Mean number of radii between legs	*n*
I–I	27.8 ± 7.9 c_1	29	1.2 ± 0.95 d_1	100
I–II (ipsilateral)	16.4 ± 6.4 c_1	58	0.32 ± 0.63 d_1	100
II–III (ipsilateral)	66.7 ± 12.5 c_2	58		
III–IV (ipsilateral)	55.0 ± 7.9	58		
IV–IV	55.0 ±7.2 c_2	29		
III – long axis body	89.5 ± 9.1	58		

Lines grasped by different legs (frequency)

Leg	Radius in free zone	Radius in sticky spiral zone	Radius in hub	Hub loop	Hub edge hole	No line	*n*
I	55	2	0	1	0	0	58
II	52	0	6	0	0	0	58
III	0	0	21	30	4	1	56
IV	0	0	34	14	7	0	55

Positions of other parts of body ($n = 29$)

	Under central hole	Edge hole	Hub or beyond
Tip of abdomen	29	0	0
Abd/ceph. junction	5	2	21

angles between her ipsilateral legs I and II were smaller than those between her two legs I (Table 1). The wide angles between the posterior legs might seem likely to reduce the spider's ability to discriminate the directions of prey hitting the rear portion of the orb. Nevertheless, the spider's responses were relatively precise, even when prey hit the web in these less well-covered positions to the rear.

Additionally in contrast to the consistent positioning of legs I and II on radii, legs III and IV held a variety of lines, including hub lines as well as (more frequently) radii within the hub (Table 1). The variety of lines grasped by legs III and IV and of the connections between them emphasizes the apparent lack of difficulty that spiders had in sensing the location of prey with these legs. For instance, longitudinal vibrations on a radius would displace a leg III holding a hub line toward and away from the spider less than if the leg were holding the radius itself. Nevertheless, the spider obtained enough information to execute precisely oriented turns, even

Table 2.—Means ± standard deviations of duration of the movement (s) of each leg between sites where it grasped lines (A), and of recognizable searching movements during this process (B) for different legs in different treatments. Numbers followed by the same letter and number in the same row differ significantly in Mann-Whitney *U* Tests.

	Treatment		
	Control	3 Radii cut	All but 5 radii cut
A. Movement between sites			
LI	0.051 ± 0.009	0.210 ± 0.242	0.116 ± 0.095
LII	0.051 ± 0.09	0.242 ± 0.207	0.165 ± 0.257
LIII	0.055 ± 0.025	0.077 ± 0.054	0.077 ± 0.046
LIV	0.077 ± 0.058	0.065 ± 0.07	0.097 ± 0.109
TI	0.07 ± 0.02	0.276 ± 0.424	0.159 ± 0.150
TII	0.05 ± 0.009	0.069 ± 0.054	0.167 ± 0.154
TIII	0.048 ± 0.035	0.045 ± 0.024	0.07 ± 0.08
TIV	0.05 ± 0.05	0.048 ± 0.022	0.08 ± 0.088
B. Searching movements at the new site			
LI	0.005 ± 0.002 c1c2	0.18 ± 0.22 c1	0.094 ± 0.14 c2
LII	0.0053 ± 0.002 c1c2	0.13 ± 0.20 c1	0.14 ± 0.18 c2
LIII	0.009 ± 0.005 a1c1	0.018 ± 0.012 a1	0.041 ± 0.087 c1
LIV	0.016 ± 0.018	0.028 ± 0.032	0.068 ± 0.102
TI	0.007 ± 0.003 b1c1	0.12 ± 0.14 b1	0.17 ± 0.15 c1
TII	0.011 ± 0.018 c1	0.058 ± 0.091	0.13 ± 0.13 c1
TIII	0.026 ± 0.043	0.008 ± 0.012	0.065 ± 0.22
TIV	0.012 ± 0.016 b1	0.053 ± 0.097	0.042 ± 0.044 b1

Table 3.—Mean rank for each leg for the order (1–8) in which they were first moved (A) and in which they seized new lines (B) when the spider turned at the hub.

	A. Order in which the first movement of each leg occurred			B. Order in which seized new line		
Leg	Control	3 Radii cut	All but 5 radii cut	Control	3 Radii cut	All but 5 radii cut
LI	1.0	1.15	1.32	1.8	3.85	2.92
TI	3.0	2.85	3.36	4.15	4.55	5.04
LII	1.45	1.4	1.36	1.95	5.1	3.0
TII	4.85		3.76	4.35	3.05	4.92
LIII	5.9	5.1	4.72	4.45	4.45	4.40
TIII	3.4	3.15	2.64	2.2	1.70	2.64
LIV	6.8	7.05	7.04	5.75	4.85	5.44
TIV	8.0	7.9	7.84	7.82	7.0	6.88

Table 4.—Percentage of times that different legs were used to jerk the web simultaneously after turning at the hub.

	LI TI	LI LII TI TII	LI TI LII	LI LII	LI	LII	*n* (jerks)	*n* (turns)
Control	24	14	57	0	5	0	21	21
3 Radii cut	47	36	2	12	1	1	149	70
All but 5 radii cut	33	5	1	25	19	18	85	30

when the legs likely involved in the orientation were relatively far apart and their placements on lines at the hub were inconsistent. The implication is that the reasons for particular leg positions at the hub probably include functions, such as supporting the spider and providing motive force to allow it to turn, in addition to sensing the site of impact of the prey. On the other hand, sensing vibrations is important, and the spiders's preference for grasping intact rather than broken radii with legs I and II while resting at the hub may function to improve her ability to sense prey vibrations with these legs.

Lines grasped by the tarsi of legs III and IV as the spider rested at the hub were more often pulled out of line than lines grasped by other legs, indicating that legs III and IV sustained an important portion of the spider's weight as she rested at the hub. The two legs IV and the leading leg III probably also provided much of the motive force used when the spider turned to attack a prey. The coordination of the movements of legs III and IV (leg TIV did not release its hold until leg LIV had grasped a new line; LIV did not release its hold until TIII had grasped a new line - Fig. 5) supports the idea that legs III and IV are especially important in supporting the spider's weight.

Responses to experimental modification of the web.—The two experiments in which radial lines near the hub were experimentally removed resulted in variable effects. Some aspects of turning, especially those involving posterior legs, were little affected. This is perhaps not surprising, because the line grasped by these legs was not altered. In contrast, the behavior of three of the anterior legs (especially LI, LII, TI) was greatly altered in these experiments, and they took much longer to find and grasp radii (Fig. 5). Probably this was because the lines these legs would have grasped were altered in the experiments. After performing small exploratory "J" movements with at least some of her legs LI, LII, and TI, the spider switched to large exploratory sweeps that were better designed to encounter more widely spaced lines. We interpret the switch from small "J" to large-amplitude waving movements on experimental webs to indicate that the spider, after failing to find the lines she expected to find, switched to the more usual exploratory behavior that is used at sites where the densities of lines are not predictably high. In other words, spiders on orbs somehow anticipated that lines would be common in the areas to which they swung their legs I and II. The persistent large searching movements of *L. mariana* resembled the persistent searches by the araneid *Neoscona nautica* (Koch 1875) when radii were experimentally removed during radius construction (Hingston 1920); presumably the spider's persistence in both cases was due to expectations that lines would be present in the area where it was searching. Experiments of this sort can open small windows on the mental processes of orb weavers.

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SHORT COMMUNICATION

Phytochemical cues affect hunting-site choices of a nursery web spider (*Pisaura mirabilis*) but not a crab spider (*Misumena vatia*)

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Abstract. Predaceous arthropods such as spiders are often adapted to hunting sites where their hunting success is greatest. We investigated the responses of two spiders to phytochemical cues that they potentially experience while hunting on leaves or flowers, and how these cues could influence their decisions where to forage. We compared the behavior of two sit-and-wait predators, *Pisaura mirabilis* and *Misumena vatia*, which hunt predominantly in the vegetation or on flowers, respectively. In choice tests, *P. mirabilis* frequently preferred leaves and leaf extracts to flowers and floral extracts and avoided substrates treated with the common floral scents β-caryophyllene and nerolidol (sesquiterpenes) in natural concentrations. In contrast, *M. vatia* did not show any preferences for any of the substrates and treatments offered. The lack of responses by *M. vatia* contrasts with earlier studies on another crab spider species (*Thomisus spectabilis*) that used phytochemical cues as a guide to rewarding flowers. The avoidance of many flowers, their extracts, and the floral scent compounds by *P. mirabilis* suggests that these cues may prevent the visitation by this and other generalised predators that potentially decrease the pollination success of a plant.

Keywords: Deterrence, optimal foraging, secondary metabolites

An underlying assumption of many optimal foraging models is that animals are behaviorally, morphologically and physiologically adapted to maximize their net rate of energy intake (Schoener 1971; Cowie 1977). A behavioral adaptation of predaceous animals is to choose foraging patches that are frequently visited by prey or to which the animals are best adapted (Krebs et al. 1974; Shafir & Roughgarden 1998). Some crab spiders, for example, show adaptations as sit-and-wait predators on flowers: they are able to change color for camouflage and enhance the attractiveness of flowers for pollinators due to their ultraviolet contrast against petals (Heiling et al. 2003). The high specialization on flowers by crab spiders is also reflected in a relatively narrow prey spectrum, which is limited to common flower visitor taxa (Nentwig 1986). Other non-web-building spiders hunt or ambush predominantly in the vegetation and thus capture a broader spectrum of prey taxa (Nentwig 1986). To benefit from their adaptations to different plant structures (vegetative versus reproductive) or to specific visitors of these structures, spiders need to perceive and thus recognize those structures. Heiling et al. (2004) have shown that crab spiders (*Thomisus spectabilis*) use visual and olfactory flower cues for patch choice.

We experimentally tested for substrate choice behavior and a role of phytochemicals in two non-web-building spiders that utilize different plant parts as hunting sites: the crab spider *Misumena vatia* (Thomisidae), which typically sits and waits on flowers to catch flower visitors, and the nursery web spider *Pisaura mirabilis* (Pisauridae), which hunts in the vegetation. In concordance with their lifestyle, we expected *M. vatia* to be attracted to flower cues, while *P. mirabils* may prefer leaves.

Between June and August 2008, we caught *M. vatia* and *P. mirabilis* spiders on fallow lands in Würzburg, Germany. We collected fifty-eight individuals of *M. vatia* on flowers of *Achillea millefolium*, *Aegopodium podagraria*, *Leucanthemum vulgare*, *Saponaria officinalis*, *Solidago canadensis*, *Trifolium pratense*, *Tripleurospermum maritimum*, while we collected all but one of 41 *P. mirabilis* from the vegetation (one individual was collected from an *Achillea millefolium* flower). We kept the spiders individually in small plastic containers in a climate chamber under long day conditions (day:night = 14:10 h, 26:19° C) and fed them with flies twice a week and continuously provided water as a small drop. We picked the plants used for the laboratory experiments in the same area.

In pair-wise choice tests, spiders were able to choose between different substrates including flowers vs. leaves of the same plant species (Experiment I), filter papers with extracts of flowers vs. extracts of leaves of the same plant species (Experiment II) and filter papers treated with synthetic floral scent compounds vs. unscented controls (Experiment III). The principal setup of these experiments (I–III) was the same: we placed individual spiders on pieces of cork representing "islands" (ca 30 cm^2) in water-filled bowls, preventing spiders from escaping. On each of these islands, we attached two wooden sticks (height = 140 mm, diam. = 3 mm) in an upright position and attached the different substrates used in the tests to the tip of these sticks. The distance between the substrates (ca 1 cm) was chosen to be close enough that the spiders could freely change between the substrates without descending to the islands but large enough that spiders were forced to make a choice. Neon lamps from above illuminated the whole setup. After spiders were placed on the islands, we observed them for 1 h, recording their position on either substrate every 3 min. We used individual spiders for several tests but not repeatedly for the same treatment.

Experiment I: We placed freshly picked flowers and leaves from *Achillea millefolium*, *Centaurea cyanus*, *Tanacetum vulgare* (all Asteraceae), *Medicago sativa* (Fabaceae) and *Saponaria officinalis* (Caryophyllaceae) in small water-filled vases. The vases were 1.5 ml standard microcaps, and we attached them on top of the wooden sticks. In each pair-wise test (flower vs. leaf of the same plant species), we adjusted the number of leaves and flowers or inflorescences so that both substrates represented approximately the same area, providing sufficient space for spiders to sit on.

Experiment II: We used the same five plant species to prepare leaf and flower extracts. We placed freshly chopped plant material into an extraction thimble and continuously extracted it with 50 ml *n*-hexane in a Soxhlet apparatus for three hours at a temperature of 85° C

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Table 1.—Generalized linear models (GLM with quasibinomial error distribution) of the proportional choices for flowers, flower extracts or synthetic compounds in *Pisaura mirabilis* and *Misumena vatia*: a) trials using fresh plant material (flowers versus leaves, Experiment I) and extracts of flowers *versus* extracts of leaves (Experiment II). Factor "treatment" refers to trials using fresh plant materials or extracts thereof. b) Trials using synthetic scent compounds versus the acetone-only treatment (Experiment III). Starting with the full model containing all explanatory parameters, each reduced model was compared with the previous one with a X^2 test resulting in deviance, number of degrees of freedom (df_1), residual degrees of freedom (df_2) and significance (P) for each parameter.

Parameter	Deviance	df_1	df_2	P
a)				
Spider species * plant species * treatment	4.53	9	288	0.58
Treatment	0.00	1	297	0.99
Spider species * plant species	5.48	4	298	0.053
Plant species	7.95	4	302	< 0.01
Spider species	14.25	1	306	< 0.001
Residual error	199.85			
Total	232.06			
b)				
Spider species * substance * concentration	2.54	3	226	0.27
Concentration	0.08	1	227	0.73
Spider species * substance	5.41	5	232	0.14
Substance	8.94	5	237	0.014
Spider species	4.37	1	238	< 0.01
Residual error	163.61			
Total	184.94			

(Baysal & Starmans 1999). We removed the solvent under vacuum and resolved the extract in acetone. We determined the volume of acetone as 0.75 · g dry weight · 200µl acetone and applied aliquots of the extract (200 µl) on round filter papers (diameter = 35 mm) that were attached on top of the wooden sticks. Thus, the extract was applied to filter papers with a mass of 75% of the plant dry weight to account for losses of the extract during the process. We tested flower and leaf extracts of each plant species again pair-wise.

In order to determine those compounds in the extracts that frequently occur in flower and leaf scents (Knudsen et al. 2006), we analysed the extracts using a Varian 3800 gas chromatograph (GC) fitted with a 1079 injector and a ZB-5 column (5% phenyl polysiloxane; length, 60 m; inner diameter, 0.25 mm; film thickness, 0.25 µm; Phenomenex) and a Varian Saturn 2000 mass spectrometer. We placed 1 µl of the samples into a quartz vial in the injector port of the GC by means of the ChromatoProbe kit (Amirav & Dagan 1997). The injector split vent was opened, and the injector was heated at 40° C to flush any air from the system. After 2 min, the split vent was closed and the injector heated at 200° C min^{-1}, then held at 260° C until the end of the run. The split vent was again opened after 4.5 min. Electronic flow control was used to maintain a constant helium carrier gas flow rate (1.0 ml min^{-1}). The GC oven temperature was held for 4.5 min at 40° C, then increased by 6° C min^{-1} to 300° C, and held for 15 min at this temperature. Mass spectra were taken at 70 eV with a scanning speed of one scan per second from m/z 30 to 650. We analyzed the data as described elsewhere (Dötterl et al. 2009), and used an internal standard (3-chloro-4-methoxytoluene) for quantification.

Experiment III: Since we expected that the phytochemical cues to which spiders respond are not specific to certain plant species, we used commonly occurring flower and leaf scent compounds that were also present in the extracts for subsequent bioassays. Among the compounds identified in the samples, we selected benzaldehyde (benzenoid), 1-hexanol, *cis*-3-hexen-1-ol, *cis*-3-hexen-1-yl acetate (all aliphatics), limonene, linalool (monoterpenoids), β-caryophyllene and nerolidol (mixture of cis- and trans-isomers, sesquiterpenoids), because these compounds are common and widespread floral scent compounds (Knudsen et al. 2006). 1-hexanol, *cis*-3-hexen-1-ol and *cis*-3-hexen-1-yl acetate are also common green leaf volatiles (Pare & Tumlinson 1999); *cis*-3-hexen-1-ol and *cis*-3-hexen-1-yl acetate were tested with *P. mirabilis* only. We dissolved substances in acetone and applied them in different amounts starting with 0.01 mMol per filter paper. In cases where a substance affected the choice of one of the spider species in this initial concentration, we subsequently reduced the amount (0.005, 0.0025, and 0.00125 mMol per filter paper) in order to explore concentration-dependent effects. We attached the scented filter papers (treatment) and filter papers treated only with acetone (controls) on top of the wooden sticks. After approximately 10 min, after the solvent had evaporated, a trial started.

Each trial (1-h period) yielded up to 20 observations from which the proportion of observations on flowers (Experiment I), flower extracts (II) or scented filter papers (III) was obtained, disregarding observations during which the spider was not present on one of the substrates. Some spiders spent time on the islands, while others did not leave it during the entire period (*P. mirabilis*: 3.0% of all trials, *M. vatia*: 7.3%); these rare events were not included in the calculation of the proportion. We performed generalized linear models (GLM) with quasibinomial error distribution (accounting for the overdispersed data) in order to explore the parameters influencing the spiders' choice. We analysed the tests with fresh plant material (Experiment I) and extracts (Experiment II) in one GLM, with the proportion of observations on flowers or flower extracts as response variable and spider species, plant species and treatment (i.e., fresh plant material or extracts) as explanatory variables. In the GLM for tests with floral scent compounds (Experiment III), we used spider species, substance and concentration (mMol) as explanatory variables. Beginning with the full model, we reduced the models stepwise and compared them to the previous one with a X^2 test (Crawley 2005). Prior to the stepwise statistical analysis, we compared the full model to a null model (model with no explanatory variables) to validate the overall effect of the combined parameters. We tested individual parameters only if the full model had significantly more explanatory power than the null model (see Mundry & Nunn 2009). Additionally, we individually tested the proportions against the null hypothesis (assuming equal visitation of both treatment and control; i.e., proportion = 0.5) with a Wilcoxon test. All statistical analyses were performed using R 2.4.0 (R Development Core Team 2009).

In 93.3 and 97.5% of all trials with *M. vatia* and *P. mirabilis*, respectively, the spider chose one of the substrates within the first

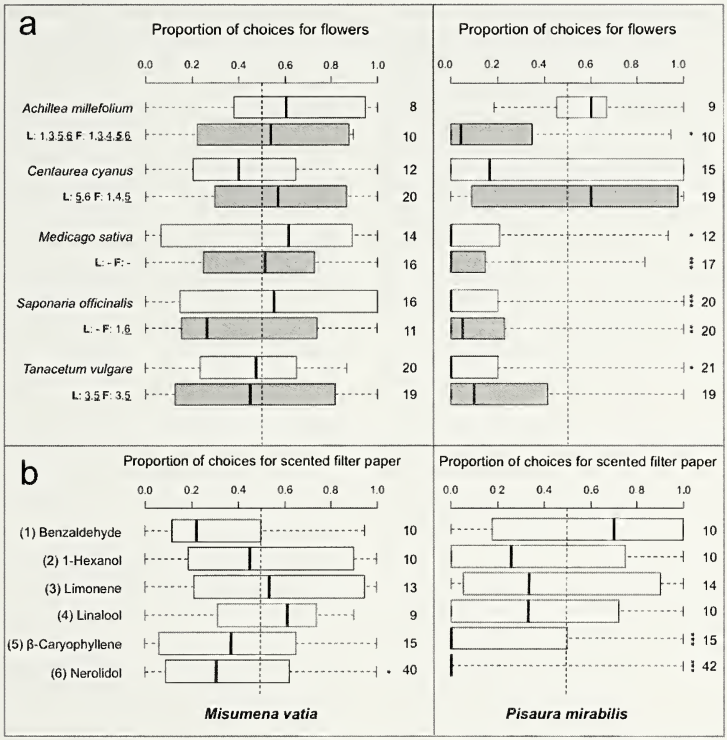

Figure 1.—Dual choices of *Pisaura mirabilis* and *Misumena vatia* between flowers and leaves, extracts or synthetic compounds. Choices were measured as proportion of choices for flowers and their extracts (a. experiments I and II) or scents (b. Experiment III) of the total time on both treatments. Significant deviation from an equal proportion of visits on flowers and leaves, or scent and control (i.e., proportion = 0.5) is indicated by asterisks using paired Wilcoxon rank sum test (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$). Sample sizes are given next to each box plot. a) White boxes show trials with fresh plant material (flowers vs. leaves), gray boxes flower vs. leaf extracts. Leaf (L) and flower (F) extracts often contained one or more substances used in the bioassay, which are listed below each species name. Numbers correspond to the substance code below (see b). Concentrations of substances in plant materials are labelled as follows: plain numbers: $1 \cdot 10^{-5}$–0.01 mMol g^{-1} dry weight; underlined numbers: 0.011–10 mMol g^{-1}; underlined, boldfaced and italic numbers: > 10 mMol g^{-1}. (b) Results of trials using synthetic floral scent compounds tested against the acetone-only control.

8 min. Once a spider climbed up a wooden stick, it rarely descended to islands again. While *M. vatia* often changed the substrates during the trial (3.0 ± 0.2 times, mean ± SE), *P. mirabilis* was less likely to switch, with only 0.8 ± 0.2 changes of the substrate per trial. The responses to fresh plant material (Experiment I) were usually consistent with responses to extracts of the same plant species (Experiment II) for both species of spider, but the spiders' choices between leaves and flowers differed strongly between plants (Table 1a). *P. mirabilis* strongly preferred leaves over flowers (and their extracts) in three out of five plant species, whereas *M. vatia* did not show any preferences (Table 1a and Fig. 1a).

In trials where spiders were allowed to choose between filter paper treated with scent compounds and acetone-treated filter paper (Experiment III), the choices depended on the particular substance and spider species. Overall, the concentration of the compounds did not affect the spiders' choices (Table 1b). Similar to the previous tests, *M. vatia* was less selective than *P. mirabilis* (Table 1b and Fig. 1b). *M. vatia* avoided filter paper treated with nerolidol, and *P. mirabilis* avoided both nerolidol and β-caryophyllene (Fig. 1b). *P. mirabils* behavior was not affected by the green leaf volatiles *cis*-3-hexen-1-ol and *cis*-3-hexen-1-yl acetate ($V \leq 50.5$, $P \geq 0.37$, Wilcoxon test). Large amounts of nerolidol occurred in floral extracts of *S. officinalis*, and β-caryophyllene in *A. millefolium*. These substances may have triggered the preference of *P. mirabilis* for leaves and leaf extracts in *S. officinalis*, and for leaf extracts of *A. millefolium* over the respective flowers or floral extracts (Fig. 1). Living flowers of *A. millefolium* were not avoided by *P. mirabilis*, suggesting that some substances were dissolved from the plant tissue and were thus present in the extracts that were not emitted by fresh plant material or were emitted in a lesser amount.

The results of our study imply that *P. mirabilis* perceive phytochemical cues and use them to decide where to ambush for

prey. In *M. vatia*, behavioral responses to these cues were much less pronounced, and the crab spiders only responded weakly to the sesquiterpene nerolidol. We had expected that *M. vatia* would prefer flowers and their extracts over leaves and their extracts, since other crab spiders (*Thomisus spectabilis*) positively responded to floral odors (Heiling et al. 2004). Crab spiders including *M. vatia* were shown to prefer fully open and functional flowers (anthesis) over senescent ones (Chien & Morse 1998; Heiling & Herberstein 2004a) and therefore have the same preferences as pollinators and use olfactory in addition to visual cues (Heiling et al. 2004). However, we could not confirm positive responses to floral odors or compounds thereof. Greco and Kevan (1994; 2001) also reported no discrimination between leaves and flowers by the same spider species. It was shown that *M. vatia* remains longer on flowers that are frequented by pollinators (Chien & Morse 1998; Morse 2000a) and on flowers that they have experienced before (Morse 2000b). We used picked flowers (i.e., not the preferred state of the flowers) that were not visited by insects, which may contribute to a lack of preferences.

The preference for leaves over flowers in *P. mirabilis* may either result from an attraction to leaves or from an avoidance of flower secondary metabolites. The trials with individual substances are consistent with the latter and suggest that floral scents or perhaps other non-volatile metabolites have a deterrent effect on this spider. Plant volatiles emitted by flowers and leaves were shown to repel or deter various arthropods (Pichersky & Gershenzon 2002; Gershenzon & Dudareva 2007; Junker & Blüthgen 2008; Kant et al. 2009; Unsicker et al. 2009; Willmer et al. 2009; Junker & Blüthgen 2010). Therefore, it is likely that the floral repellence of this spider represents a typical response of a broad spectrum of generalised predators and other taxa that are not specifically adapted to flowers.

Crab spiders are predators that exploit the mutualism between flowers and pollinators and thereby have detrimental effects on pollination and consequently reproduction of plants (Dukas 2001; Dukas & Morse 2003; Heiling & Herberstein 2004b; Reader et al. 2006; Goncalves-Souza et al. 2008; Ings & Chittka 2008; Brechbühl et al. 2010). Chemical floral cues that prevented predators such as spiders and other floral antagonists from visiting flowers and simultaneously attracted pollinators would maximize the plants' reproductive success (Brown 2002; Irwin et al. 2004; Junker & Blüthgen 2008). Animals that depend on floral resources (obligate flower visitors) are able to tolerate defensive floral scent compounds and even use them as host-finding cues, while facultative flower visitors are not able to (Junker & Blüthgen 2010). The results of the present study suggest such a dichotomy, in which an obligate flower visitor (*M. vatia*) is adapted to flowers as a place to sit and wait for prey, which may include a tolerance against otherwise defensive floral compounds. In contrast, *P. mirabilis* is adapted to use the vegetative plant parts as hunting sites and may not have been subjected to a selective pressure to tolerate the same compounds.

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Reproductive behavior of *Homalonychus selenopoides* (Araneae: Homalonychidae)

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Abstract. *Homalonychus selenopoides* Marx 1891 is endemic to the coastal plains of the Sonoran Desert in the state of Sonora, Mexico and the southwestern United States. Although the species was described more than a century ago, nothing is known about its behavior. We collected spiders in the southern Sonoran Desert to study their reproductive behavior, which we recorded with an infrared camera, mainly at night. Sperm induction was of an indirect type; males wove a triangular sperm web about 2 cm^2 near the ground. Females and males prepared threads of silk and sand. Courtship behavior was intermediate between levels I and II, and the copulation position was a modification of type III, where the male tied the female's legs with silk before mating. Sexual cannibalism may occur during mating. Females began to spin their egg sac at ~11 days after mating and completed it in ~15 h, including ovipositioning. The outer layer of the egg sac contained sand, and the sac was surrounded by a garniture of cords of silk and sand, possibly to protect the eggs from desiccation and as a barrier to parasites and predators.

Keywords: Sperm induction, courtship, copulation, egg sacs

Homalonychus selenopoides Marx 1981 is endemic to southwestern Arizona and small areas in southern Nevada and California. In México, it occupies the coastal desert plains in the state of Sonora and Isla Tiburon (Roth 1984; Crews & Hedin 2006). Despite its broad distribution, and more than a century after it was first described (Marx 1891), virtually nothing is known about its behavior. This species is included in the family Homalonychidae, which is represented only by the genus *Homalonychus* Marx 1891, including two species. The other species, *H. theologus* Chamberlin 1924, inhabits the Baja California peninsula, extreme southeastern California, and southern Nevada. Homalonychids are cursorial spiders that are not commonly encountered (Vetter & Cokendolpher 2000); they are nocturnal and conspicuous. Adult males are 6.5–9.0 mm, and adult females are 7.0–12.8 mm and are usually found in fine sand or soil and under rocks, wood, or debris. Typically, juveniles and adult females camouflage their bodies with fine soil particles that adhere to the setae of their integument, which allows the spider to blend in with the surrounding soil (Duncan et al. 2007). They are often found slightly buried in the sand with their legs extended (Roth 1984).

Gertsch (1979) mentioned that the family Homalonychidae was enigmatic because very little was known about it. Even now, there are few studies available. Roth (1984) carried out systematic studies of the family, Vetter & Cokendolpher (2000) described the egg sac and defensive posture of *H. theologus*, and Domínguez & Jiménez (2005) reported on sexual and cryptic behavior of *H. theologus*. Crews & Hedin (2006) explained the phylogenetic divergence of the two species and Duncan et al. (2007) described the convergence of *Homalonychus* and *Sicarius* Walckenaer 1847 (Sicariidae) in the morphology of their setae for retaining soil particles. Other studies (Roth 1984; Griswold et al. 1999; Miller et al. 2010) are concerned only with the systematics or phylogeny of homalonychids.

Here, we describe the reproductive behavior of *H. selenopoides* under laboratory conditions, including sperm induction, preparation of silk threads with adhering sand, courtship and copulation, and spinning of the egg sac.

METHODS

We collected spiders in the bed and sloping sides of the ephemeral stream El Macapul and surrounding area located northern of San Carlos, Sonora (27°59′00″N, 111°02′16″W and 28°00′55″N, 111°03′05″W), in the extreme southern part of the Sierra El Aguaje. The climate is very dry: hot in summer and warm in winter. The mean annual temperature is 22–24° C and the mean annual rainfall is 75–200 mm; summer and winter rainfall is split ~ 90% and ~ 10%, respectively (INEGI 1999). Vegetation is desert scrubland with *Bursera* and *Jatropha* predominating (INEGI 1984). Soils are weakly developed and shallow (< 25 cm), usually composed of unconsolidated coarse-textured sand and fine gravel with rocky areas without soil or some soil found in depressions among the rocks (INEGI 2002). The stream bed is almost entirely sand and gravel.

We made 17 diurnal collections with 3–4 participants between October 2007–April 2008. During this period, we captured 186 adult and immature spiders from under stones, dry cattle dung, wood, bricks, or cardboard. We placed each live spider individually in a plastic container and transported all of them to the laboratory in Hermosillo, Sonora, Mexico. Male and female voucher specimens were preserved in 75% ethanol and deposited in the Arachnological and Entomological CIBNOR Collection in La Paz.

We maintained each live spider individually in a 500-ml transparent plastic jar containing 1 cm soil substrate from the collection site and a small container of wet cotton for water. Specimens were initially fed crickets (Gryllidae) and cockroaches (*Blattella* sp.), and later mealworm larvae *Tenebrio* sp.

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(Domínguez & Jiménez 2005). We used mealworms because they are easy to cultivate. The breeding room (3 × 3 m) was kept at 18–28° C, under natural photoperiod, and 36–60% relative humidity. We observed courtship and copulation in this facility, but made observations of sperm induction and spinning of egg sacs in another small room. We recorded spider behavior with an 8 mm digital camcorder equipped to record infrared light.

Sperm induction.—We placed five males reared in the laboratory and two field-collected males individually in 1750-ml clear plastic jars (13 cm diameter) with fine sand to a depth of 2.5 cm. We added a small flat stone for attachment of the sperm web, as well as an arched cardboard shelter and a small container of wet cotton. From 14 March–14 April 2008 from 20:00–08:00 h, we made momentary observations at intervals of 20 min using an infrared light camera. For these specimens, the ambient temperature was 17.2–30.7° C, natural photoperiod, and 20–47% relative humidity.

Mating behavior.—From January–March 2008, we formed 25 mate pairings with eight adult males and 23 adult females collected in the field (age and reproductive status unknown). Because we had few males that were very variable in their behavior, we used mainly males that were actively searching in these trials; the other males were less active or fled from females. Throughout July 2008, we formed another 20 pairings with 14 males and 12 females reared in the laboratory, (virgins, of known age) plus one female from the field. In these trials, we made these pairings at random, although the males were also variable in behavior. We formed additional mating pairs (one in October 2008 and 18 in July–August 2009) to see if additional behavioral acts had been undetected during the initial pairings; these results were not used in statistical analyses. In all these cases, some females and, more frequently, males were used again to form new pairings. Observation schedules and laboratory conditions were as follows: in January 2008, 14:30–18:00 h, 18–19° C, 50–60% relative humidity; in February 2008, 15:00–20:00 h, 24–25° C, 50–60% relative humidity (temperature was maintained with an electric heater); in July 2008, 20:00–23:00 h, 24–28° C, 36–55% relative humidity. We placed individual females in glass terraria (20 × 20 × 10 cm) containing a 2-cm substrate of fine sand. We introduced a male 20 to 177 min later (median = 72 min). If the female was receptive, we filmed the behavior and continued filming for 15 min after copulation. We separated individuals or changed their partners if copulation failed to occur within 55 min, or sooner, if they tried to escape, or if an individual repeatedly ran from its partner or assumed a defensive posturing of paired legs. When disturbed, these spiders extend their first two pairs of legs together and forward and the last two pairs together and backward (Vetter & Cokendolpher 2000). In one trial in July 2008, we introduced two males simultaneously.

Egg sac construction.—We used 20 captured adult females, each of unknown reproductive status but with a large opisthosoma, to observe egg sac spinning. These females were captured in the winter of 2008. We placed each female separately in a 1750-ml transparent plastic jar containing a 3-cm sand substrate and one of three types of shelters: 1) an arched piece of cardboard; 2) flat stones glued together with molding silicone; or 3) stones with a glass ceiling. Shelters 2 and 3 had a flat horizontal roof of at least 5 × 5 cm at a height of 2.0–2.5 cm above sand level. We placed five females in these terraria, replacing them every 4–5 days if they failed to spin an egg sac. Observations lasted from 22 April–16 May 2008. Ambient temperature was 24.8–33.8° C, with natural photoperiod, and 16–31% relative humidity. We did not observe or record the spinning of the egg sacs by females that had copulated in the laboratory in July 2008; however, we noticed that each female had produced several egg sacs.

Figure 1.—*Homalonychus selenopoides* male during loading of sperm.

RESULTS

Sperm induction.—We observed the entire sperm induction process once (02:38–03:00 h), when a male wove a sperm web in 5.9 min, close to the sandy substrate; it was slanted and attached to the cardboard shelter and to the wall of the jar. The male stood on the substrate, placed his body on the web, and pressed against it twice. Infrared light failed to show sperm deposition. Subsequently, the male moved a pedipalp in an arch-like motion from top to bottom on one edge of the web to load the pedipalp with semen, rubbing the ventral part of the cymbium against the lower surface of the web (Fig. 1) with soft movements. He raised this pedipalp to carry out the same process with the other pedipalp. So, the semen was deposited on the upper side of the web and it was then absorbed through to the underside. This stage took 7.8 min. The male then climbed off the web and rested on the sandy substrate. The entire induction process took 16.5 min. We also observed the last 2 min of semen loading of another male at 04:28 h, with a position and process identical to the one that we had observed in its entirety. This male then rested on the web for 2.2 h.

Three laboratory-reared males (age 6–8 days as adults) and two field-collected males wove six sperm webs (one in November 2007 and five in March–April 2008). Web dimensions varied from 9 × 13 × 15 mm to 21 × 26 × 28 mm. Webs were triangular, thin, and semi-transparent, with one or several layers of silk (Fig. 2). Webs had two strips of denser sheets that extended from the center to one edge; on this edge, the male arched his pedipalps during induction. The

Figure 2.—Sperm web of *Homalonychus selenopoides.*

Figure 3.—Thread of silk and sand spun by a *Homalonychus selenopoides* male.

webs were set between stone or cardboard and the wall of the jar, inclined at angles of 40–70°, with a height above ground level at their lowest between 2–8 mm and at their highest between 12–24 mm. One male wove two sperm webs, another male wove over a prior web, and two males wove rectangular webs.

We observed variations in form and size of other male webs, but these were not observed during construction. Two males wove triangular ~1 cm-wide sperm webs attached to the top of the container and the mesh. Other males wove webs on the sand that were 1–2 cm long, as short strips that went from "aggregates" of sand and silk from the ground, stuck to the wall of the jar or the cardboard shelters. Some spun elongated silk sheets (~ 1 × to 5.5 cm) upon sandy aggregates. Other males first wove smaller webs before undertaking larger sperm webs.

Silk and sand threads.—In July 2008, five males placed in glass terraria spun six threads of silk and sand in form of "cords" (Fig. 3). Three threads were spun before and two after copulation, and another was spun without the spider participating in copulation. Males spun threads with their spinnerets, moving slowly with their legs close to their body and constantly touching the thread with their pedipalps. They walked very close to the floor, weaving in the same track two or even five times. The spiders spun threads in 4.7–18.3 min. Four of the threads ranged from ~ 8.0–17.3 cm, with knobs or swellings at one or both ends. Two threads were 1.9 and 2.4 cm long, with one thick end and the other end bifurcated. We did not observe reactions of females to male threads, because the males approached the female to mate before the females walked on the threads. In July 2009, one female spun threads with silk and sand prior to copulation. The female continuously wove these threads with her spinnerets, leaving a grid of threads on the sand. The threads were very thin in the form of a rosary, but were visible because the sand grains adhered to them. The male placed in this terrarium encountered the female's threads and immediately began spinning a thread (cord).

Mating behavior.—We observed 16 successful pairings, three in January–March 2008 and 13 in July 2008. Two pairs of spiders copulated twice; these second matings were not considered in our analysis. Sexual behavior was divided into three stages: pre-copulation, copulation, and post-copulation (González 1989; Domínguez & Jiménez 2005). The sequences of behavioral acts and transition frequencies, including secretion of silk and sand threads, are summarized in Fig. 4.

Pre-copulation: During his search to find the female, the male advanced in what appeared to be a random manner, exploring, walking slowly, and gradually raising and lowering his first pair of legs. The male could also approach the female directly in a targeted manner when he apparently had identified her. In 16 observed copulations, search time prior to mating ranged from 0.1–39.4 min (median = 11.8 min). The initial contact or touch between potential partners was with the tarsi of the forelegs. When the male reached a receptive female, she became passive and he quickly and repeatedly touched and tapped her prosoma, opisthosoma, or legs with the tarsi of his forelegs for ~ 1–3 s. If the female was initially unreceptive, she could abruptly retreat or walk away. Then the male initiated the courtship. Females also initiated approaches or courtship; then the male could flee or begin tapping or begin courtship. Rejections in form of attacks against consorts were observed only in one pair; the female attacked the male and later the male attacked the female.

During courtship, the male drummed on the ground with his forelegs or with his first two pairs of legs. Legs vibrated when they were in contact with the ground. The left and right legs were extended and moved up and down quickly and alternately. Also, he drummed on the ground slowly and gently with the pedipalps while moving forward or side-to-side. When a female initiated courtship, she approached the male to touch him, then took a "stalking" stance while moving slowly or swiftly with one or more quick approaches. Of the observed pairings, 50% included some period of male courtship. In 25% of the 16 pairings, females approached and touched males. When it occurred, male courtship lasted from < 1–33.5 min (median = 3.1 min) and the female courtship lasted only a few seconds.

Copulation: After a male touched a female, she brought her legs toward her body, leaving the patellae almost touching above the carapace; only the tarsi and metatarsi of the fourth pair of legs were directed backward. The female remained passive and motionless in a quiescent state (Becker et al. 2005). The male climbed onto the body of the female, tapping her with the tarsi of the forelegs and pedipalps anywhere on the body and legs. Then the male climbed up one side or the back of the female and settled on top of the female, facing the opposite direction. During mounting, the male continuously touched the body of the female. Of 16 observed copulations, in seven of the mountings (44%), males approached the females frontally; the other mountings were made from behind or from one side.

While mounted, the male wove threads of silk in circles around the legs of the female to form a broad ring tie, like a veil, covering the exposed surface of the legs, except tarsi and metatarsi of the fourth pair. The male also added sand to the silk on the sides and bottom of the female body as "counter balances." This web is known as the "bridal veil" (Bristowe 1958; Domínguez & Jiménez 2005). While the male was weaving, he was tapping the female's body and legs with his forelegs and pedipalps. The tying was repeated alternately and successively with insertions of the pedipalps (a tying always preceded insertion of a pedipalp).

During insertions of the pedipalps, the male placed the quiescent female on her side, either right or left, moving to that side while he was embracing her with his first three pairs of legs and resting with the fourth pair on the floor. The male's left pedipalp was inserted into the genital opening of the female on the left side while the female was lying on the right side or vice versa. The pedipalps could be alternately inserted, or a pedipalp could be sequentially inserted. During insertion of the pedipalp, the male vibrated his legs II and IV on the same side as the inserted pedipalp. In the 16 observed pairings, the duration of copulation (mounting) ranged from 0.6–9.4 min (median = 1.9 min). The number of pedipalp insertions per mating ranged from 2–12 (median = 2.5); of 85 individual insertions, 66% were done with the right pedipalp and 34% with the left pedipalp.

Successful mating among pairs depended on the origin of the females. Of the 25 pairs formed with the field-collected females in January–March 2008, the successful rate for mating was 12% because only three pairs mated; thus 88% of the females were unreceptive. One female copulated twice with the same male during the same session. On the other hand, the rate of success of the 20 pairs formed with virgin laboratory-reared females in July 2008 was 65%. There were 12 ordinary copulations and one case in which a female presented with two males, mated first with one, then minutes later copulated twice with the other. Five of 12 virgin females received a second or third partner after rejecting the previous male, but finally 100% of the virgin females were receptive. The only pair that included a field-collected female did not copulate.

Post-copulation: Copulation finished when 62.5% of the males dismounted from the females and withdrew, walking away while they remained quiescent for a few seconds. Also, copulation finished when 37.5% of the females were no longer quiescent, extended their legs breaking the bridal veil, and the males fled. Females usually took less than 2 s to break the veil and walk or run, although one female took 16 s and one took 10 min.

After breakout, females rubbed their legs together to remove the remnants of the bridal veil. 38% of the females dug in the ground at least one time, then rubbed and wiggled the back and belly of their prosoma and opisthosoma, and legs in the soil; sand particles then adhered to their body surface. We did not observe this behavior in males. In all pairings, males vibrated their opisthosoma after dismounting; they raised and lowered it with quick short movements. Also, the males cleaned the ventral cymbium of the pedipalps (presumably copulatory structures) with their chelicerae. These actions occurred at least one time in each male and took place within a few minutes after copulation. Males showed post-copulatory courtship in 50% of the couplings. We present the full range of post-copulatory acts and their sequences in Fig. 4.

In January–March 2008, there were two cases where the males were captured and killed by the females within the first 7 min of waiting, without courtship or mounting taking place. When males were killed, their body contents were consumed in the subsequent (undetermined) hours. In January 2008, we observed one event of sexual cannibalism after copulation. In this case, after the last insertion of the pedipalp the female suddenly extended her legs, broke the veil and quickly reached the male as he attempted to escape; all this took place in about a second. In October 2008, there was another event of sexual cannibalism, but this male was caught during mating. In this case, both individuals were lying on the ground, belly to belly in opposite directions, when the female grabbed the male on the ventral side of his opisthosoma. The female broke the veil, broke free of the male for a moment, and caught him. These males were also consumed in the subsequent hours.

In the 22 pairs that did not copulate in January–March 2008, we observed rejection by both males and females, immobility of one or both partners, with or without legs in paired position, and constant attempts to escape from the terrarium. Also, we observed that some males touched or stood on unreceptive females with their tarsi, but apparently the females were not detected. Our waiting time to complete these trials ranged from 22–55 min.

Egg sac construction.—Eight females that copulated in July 2008 started to spin their first egg sacs 9–13 days after mating; spinning was not filmed. Five females collected in the field began spinning their egg sacs, but only four finished. We recorded the spinning of two egg sacs from beginning to end and the other two after the first phase had started.

The female initiated the egg sac construction behavior when she explored the shelter roof; also, she could scratch the sandy substrate. Then she started spinning the egg sac by weaving a silk sheet, thin and circular, on the roof of the shelter. This took 54 and 69 min. Thereafter, she wove thick double strands of silk and sand in the shape of cords. While she was inverted on the ceiling of the shelter, she dropped her opisthosoma and fourth pair of legs grasping the shelter with her three other pairs of legs. With her spinnerets in contact with the sand, the female secreted silk threads and added sand to these in short zig-zag strokes, leaving a cord behind her, which was also folded in a zig-zag pattern. Afterwards, the female raised her opisthosoma and the fourth pair of legs, staying inverted, and attaching to the ceiling the proximal end of the extended cord that was attached to her spinnerets. This process was repeated with other cords to form a first outer circle or ring of the sand-

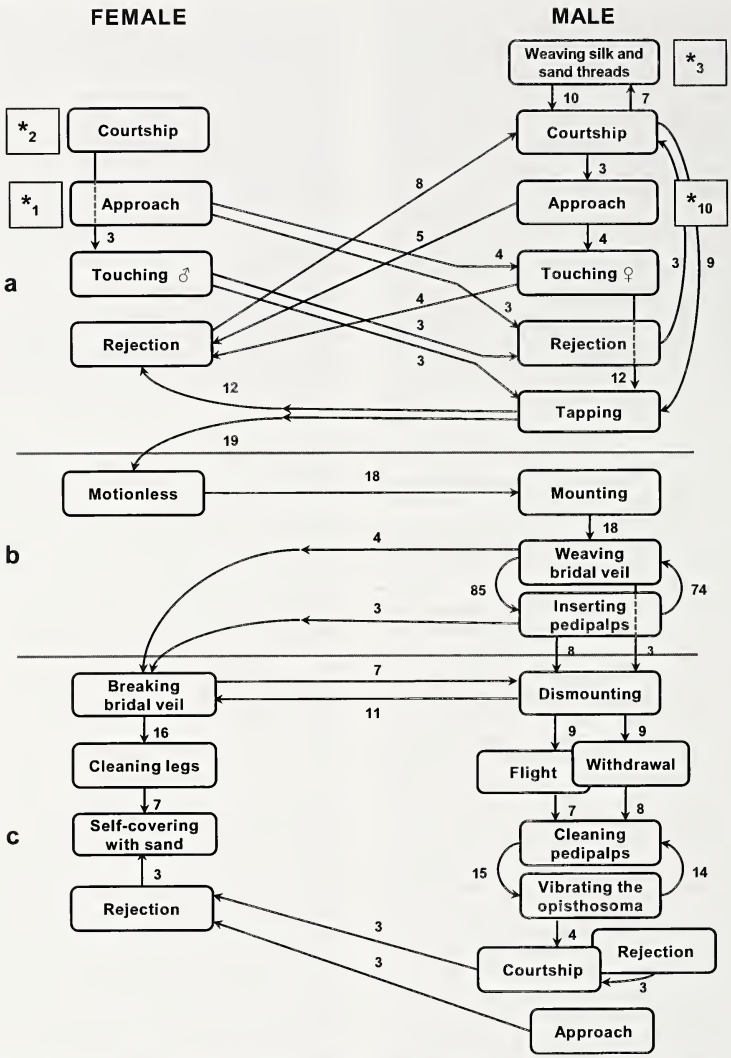

Figure 4.—Sexual behavioral sequences observed in 18 pairings of *Homalonyclus selenopoides*. a) Pre-copulatory stage; b) Copulatory stage; c) Post-copulatory stage. The numbers adjacent to arrows represent the total number of transitions. Sequences that occurred one or two times are not included. Asterisks indicate the behavioral acts where a sequence began, and the numbers beside the asterisks indicate the number of sequences that began in these acts.

silk garniture of the future egg sac. During this process, the female was centrally positioned inside this circle (Fig. 5) as she spun silk strands concentrically inward (Fig. 6). The garniture increased progressively in thickness, and the internal space was reduced to include the female only. The female lowered herself from the shelter at intervals to rest on the ground or to dig and accumulate sand taken from under the shelter.

We inferred that the females lined the interior of the last cord layer circle of the egg sac with silk because the tube walls moved continuously, forming the inner layer of the egg sac.

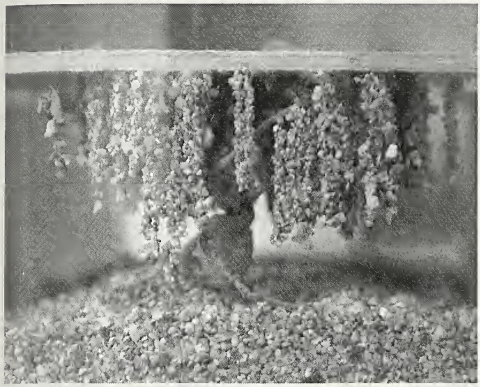

Figure 5.—*Homalonychus selenopoides* female spinning the outer ring of silk cords of the egg sac.

The lower end of the tube was gradually withdrawn and sealed, forming the completed egg sac. Afterward, females were immobile for 5–6.5 h, with only sporadic movements of the tubular wall. We inferred that oviposition occurred during this time. Subsequently, females broke the bottom side of their sacs with their first two pairs of legs to exit. Escaping required 28 s and 10.3 min for two females observed. Immediately afterwards, each female embraced her egg sac and closed the exit rupture with her spinnerets. The other two females were not observed because they were on the opposite side of the egg sacs from where we were filming. It took 14 and 15.5 h from the start of weaving the silk sheet until the females emerged from the sac.

The whole egg sac consists of two sections, a thick exterior garniture of sand-silk cords and the egg sac in the center. The whole structure is shaped like a short cylinder and the egg sac

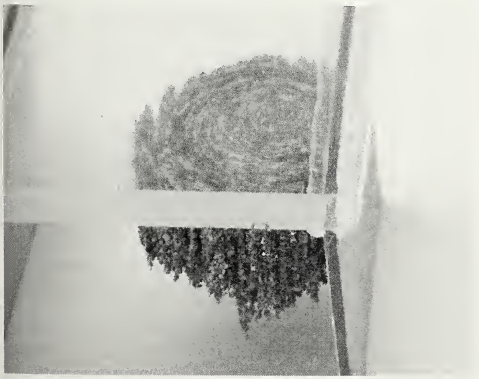

Figure 6.—Full egg sac of *Homalonychus selenopoides* showing concentric arrangement of the silk cords.

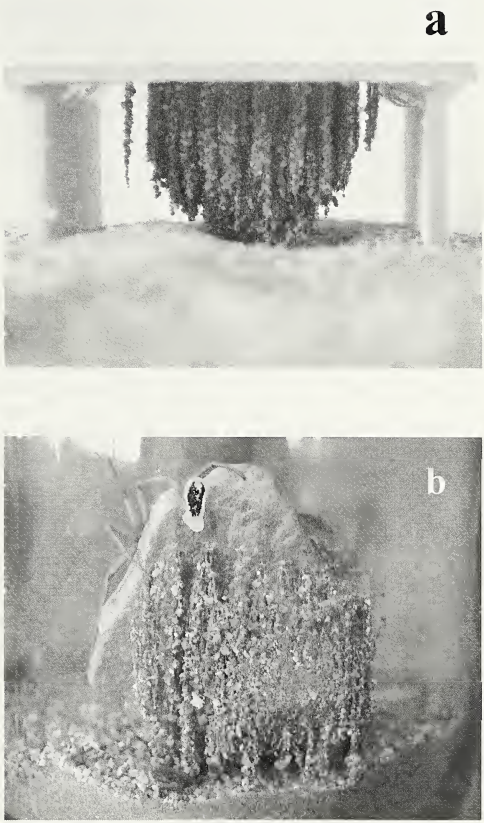

Figure 7.—Egg sacs of *Homalonychus selenopoides*. a) Egg sac spun on a wide, horizontal surface; b) Egg sac spun on a reduced, sloping surface.

can extrude from below, between the garniture of cords (Fig. 7a). Six other captive females also spun egg sacs in the laboratory. One female spun a flattened egg sac under an inclined rock in a very narrow space (Fig. 7b). Later, this female spun two other flattened egg sacs under the same rock. Moreover, in the absence of a shelter, four unobserved females deposited naked eggs directly on the sand surface and the other female also deposited naked eggs on the woven cloth that covered the jar.

DISCUSSION

We observed all stages of reproductive behavior of *H. selenopoides*. Most reports on spider reproduction include only some stages. Sperm induction had not been observed before in the Homalonychidae, and the function of the bridal veil in *H. selenopoides* still remains obscure. Apparently, adding sand to

the silk threads made by males and females and the garniture of cords of silk and sand surrounding the egg sacs spun by females only occur in these spiders. We here discuss the functional role of these features and possible phylogenetic implications of their sexual behavior.

Sperm induction.—The horizontal, triangular shape of the sperm web matches what is commonly observed in spiders (Foelix 1996). The square form is also common (Gertsch 1979). We found both web forms in different sizes, but the factors that determined the shape and size of the webs were not clear to us. Although the sperm web of the sister species *H. theologus* is triangular, its area is only 2–4 mm^2 (Domínguez & Jiménez 2005), much smaller than what we found among *H. selenopoides*. Duration of sperm induction is consistent with observed behavior of most spiders, which require less than half an hour to perform (Gertsch 1979). The filling of pedipalps with sperm corresponds to the indirect form (Foelix 1996) and is consistent with what is commonly reported for cursorial spiders (Jackson & Macnab 1991). The alternating loading of pedipalps is similar to *Schizocosa crassipes* (Walckenaer 1837) (Lycosidae), but differs in that *S. crassipes* slowly agitates each pedipalp after loading the sperm (T. H. Montgomery in Gertsch 1979). Webs were not consumed by males, as in *Sicarius* (Levi 1967).

Induction is a common phenomenon, but observing this behavior requires patience (Gertsch 1979). Reports of induction vary from only descriptions of sperm webs (Domínguez & Jiménez 2005; Sierwald 1988), partial observations of the induction process (Fraser 1987), single observation of the entire process (Levi 1967; Jackson & Macnab 1991), and repeated observations of the entire process (Rovner 1967; Stumpf 1990). When the process takes several hours, it is easier to observe, as in some Theraphosidae (Costa & Pérez-Miles 2002). The males we studied were very sensitive to light, sound, and vibration during sperm induction and if disturbed, either ceased their activity or did not initiate it. Hence, we assume that successful observations of induction depend on its duration (Costa 1975), sensitivity of the species to surrounding environmental events, and whether the induction is unpredictable or it occurs immediately before or after pseudo-copulation or copulation.

Silk and sand threads.—We were surprised to observe males and females spinning threads of silk and sand. We noted that immature and adult specimens have their spinnerets contracted in the opisthosoma and, like other cursorial desert spiders, do not create security threads. Hence, we assume that releasing threads when males and females are searching for potential mates has a role in sexual marking. The presence of sex hormones in the threads is possible because silk is the main hormonal substrate in spiders; in other species both sexes emit and respond to pheromones (Gaskett 2007). Male silk can attract females (Roland 1984) and promote the beginning of courtship (Ross & Smith 1979). This function seems reasonable for *H. selenopoides*, because it rarely occurs in the field (unpublished data). Moreover, male silk affects courtship of conspecific males (Ross & Smith 1979; Ayyagari & Tietjen 1987). We observed that a male walking on a thread produced by another male immediately stopped and wove his own thread just above the previous one. There is no precedent in the literature for this behavior or about spiders adding sand to silk threads.

The pheromones released by females spiders as an attractant for males to induce courtship are amply documented (Gaskett 2007). However, in our study, only one virgin female spun silk threads. It is possible that the small size of the terrarium permitted pairs to meet more easily than in the field, so spinning of silk threads by females (and males) was unnecessary, and these silk threads were by-passed in favor of direct contact between partners (Dondale & Hegdekar 1973). In the field, where these spiders are uncommon, silk threads could play an important role for locating mates.

Mating behavior.—In general, mating behavior of *H. selenopoides* is similar to *H. theologus*. In both species, males usually take the initiative and approach females; however, some *H. selenopoides* females made approaches and initial contact to trigger the search or male courtship. Initiative by females for courtship was not observed in *H. theologus* (Domínguez & Jiménez 2005). Females starting courtship has also been observed in *Lycosa* spp. (Costa 1975; Rovner 1968). Although *Homalonychus* females are relatively sedentary (Crews & Hedin 2006), it is possible that, in their sexually receptive stage, they are more vagile. Active participation of both sexes in search and courtship may explain their presence in pitfall traps in the collection area. 15 of 17 *H. selenopoides* specimens trapped were adult males (47%) and adult females (53%) (unpublished data).

In *H. selenopoides*, mounting occurred on either side of the female. During copulation, the males vibrated legs II and IV, in contrast to *H. theologus*, where mounting occurred frontally and males vibrated legs II and III during copulation (Domínguez & Jiménez 2005). In both species, copulation could finish when the male ceased activity, dismounted from the female, and withdrew, but in *H. selenopoides,* there was variation in the way to end copulation. In this latter species, copulation also ends when the female suddenly spreads her legs, breaks the nuptial veil, and the male has to flee.

Courtship falls between levels I and II described by Platnick (1971), as in *H. theologus* (Domínguez & Jiménez 2005), Lycosidae, and Pisauridae. Evidently, the primary trigger of courtship or mounting behavior in the male is the direct contact with the female, but we hypothesize that males can also detect a female by a chemical stimulus. We assume that there is a contact sex pheromone in the cuticle of virgin females (Dondale & Hegdekar 1973). When males touched unreceptive and motionless field-collected females in some pairs, they did not attempt mounting. But in most other pairs, when the males touched virgin laboratory-reared females, they immediately attempted mounting. Male spiders detect pheromones by touching the females because they have tarsal receptors involved in sexual recognition (Foelix 1996). Pheromones that attract or promote the courtship of males in the female cuticle have been reported in at least 25 species of spiders (Gaskett 2007). Pheromones in *Homalonychus* and their role in sexual behavior deserve to be investigated.

Homalonychus selenopoides take the "lycosid position of copulation" (position III, Foelix 1996), similar to what is described for other wandering spiders, such as Lycosidae (Stratton et al. 1996), Pisauridae (Merret 1988), Agelenidae (Fraser 1987), Philodromidae, Clubionidae, Salticidae, and Thomisidae (Foelix 1996). Basically, in this position, males mount facing the opposite direction from the female, with the

ventral surface of the male prosoma on the dorsal surface of the female opisthosoma. In lycosids, males lean towards either side of the female to insert one or another of their pedipalps. In *H. selenopoides* this position is modified. The male places the quiescent female toward one side and then the other to insert one or another of his pedipalps, similar to the report on *Ancylometes bogotensis* (Keyserling 1877) (Pisauridae) (Merrett 1988) although in *H. selenopoides* the insertion of pedipalps is not strictly alternating. After this point, copulation is identical to that of *H. theologus* (Domínguez & Jiménez 2005).

The low frequency of sexual cannibalism observed is consistent with the claim that high frequency of cannibalism is a myth and not common among spiders (Foelix 1996). The two events of sexual cannibalism here observed are the first reported for Homalonychidae, because this behavior was not observed in *H. theologus* (Domínguez & Jiménez 2005). For the other two cases of predation upon males, these events did not represent sexual cannibalism because there was neither courtship nor copulation (Elgar 1992).

Regarding success in pairings, it is possible that *H. selenopoides* females are monandrous. This would explain the marked difference in the percentage of successful copulations between females collected in the field and the virgin females obtained in the laboratory. It is likely that most females collected in the field had already copulated since we also collected adult males.

Bridal veil.—The bridal veil is defined by Bristowe (1958) as silk threads deposited by males on females during courtship or copula. Although it occurs in species of at least 12 families, the veil of *H. selenopoides* is only identical to *H. theologus* (Domínguez & Jiménez 2005). According to the brief description of the veil of *Thalassius spinosissimus* (Karsch 1879) (Pisauridae) (Sierwald 1988), the shape and width of the bundle appear to be similar to the two *Homalonychus* spp. The extent of tying is also similar to *A. bogotensis* (Merrett 1988), but in the pisaurid, the veil is composed of an outer ring at the distal end of legs I–III and an inner ring at the level of the patellae.

Several functional hypotheses have been proposed for the bridal veil (Ross & Smith 1979; Schmitt 1992; Domínguez & Jiménez 2005; Aisenberg et al. 2008). We cannot support or refute the suggestion that the veil in *H. selenopoides* functions as a deterrent to other males during copulation. However, we doubt that the veil in *H. selenopoides* aids to identify the male as a consort because the veil is woven when the female is receptive and has become quiescent, nor do we believe that the veil restrains the female to prevent her from attacking the male or inhibit the aggressiveness of the female, as suggested for *H. theologus* (Domínguez & Jiménez 2005). We observed females that quickly broke free of the veil after copulation, ending their quiescence. The female that cannibalized her partner immediately after copulation broke out and captured him in about one second. Robinson & Robinson (1973) proposed that the main function of the bridal veil in all species that produce it is to stimulate the female. Preston-Mafham (1999) argued that courtship behavior in these species is very rudimentary, but pheromones in the veil may cause important physiological changes in the female epigynum to prepare it for insertion of the pedipalps. To fully determine the role of the bridal veil in *Homalonychus* requires further investigation.

Egg sac construction.—We have not found a precedent in another genus of spiders for garnitures of silk and sand cords surrounding the egg sac as in *Homalonychus*. Although *Sicarius* attaches sand to the wall of its egg sac (Levi & Levi 1969), it does not make a garniture of cords. Because *Sicarius* spp. inhabits deserts of South America and southern Africa (Platnick 2009), Domínguez & Jiménez (2005) suggest a convergence between the two phylogenetically unrelated genera as a response to harsh desert conditions. However, there are distinct differences in the timing and egg sac spinning process, form, and structure, and the fact that *Sicarius* spp. use their legs to bury their egg sacs with sand.

The description of the egg sac of *H. theologus* (Vetter & Cokendolpher 2000) is incomplete because it fails to mention the thick exterior garniture of cords, although in a published photograph some of them are apparent. Also, spinning of the egg sac of *H. theologus* (Domínguez & Jiménez 2005) was made at an atypical site, the side wall of the container. We infer that *Homalonychus* requires a shelter with a horizontal roof for spinning typical cylindrical egg sacs with exterior garniture of silk cords. We suggest that further study is needed to define the typical structure and spinning process of egg sacs in *H. theologus*. We agree with Vetter & Cokendolpher's (2000) and Domínguez & Jiménez's (2005) hypothesis that the sand covering the egg sac acts as a protection from predators and parasites and ameliorates the intense desert summer heat, where temperatures can exceed 45° C. We suggest that the cord garniture has this function, at least.

Phylogenetic implications.—Since the genus *Homalonychus* was described in 1891, it has remained in an uncertain phylogenetic placement (Griswold et al. 1999). Historically, researchers have hypothesized that there is a relationship with Pisauridae, Selenopidae, Zodariidae, Ctenoidea, and Pisauroidea (Crews & Hedin 2006). Proposals based on morphology, sexual behavior, and even on molecular analysis appear insufficient to draw a stable phylogenetic hypothesis.

Courtship and mating behaviors are considered important characteristics for reconstructing phylogenetic relationships in spiders (Platnick 1971; Bruce & Carico 1988; Stratton et al. 1996). Based on the mating position, and occurrence and form of the bridal veil, Domínguez & Jiménez (2005) suggest that *H. theologus* is related to Pisauridae and could be included in the superfamily Lycosoidea of Coddington & Levi (1991). Based on morphological characters, Roth (1984) proposed retaining Homalonychidae as a separate family, criteria maintained by Coddington and Levi (1991). Griswold et al. (1999) lists Homalonychidae and seven other families as groups whose relationships in higher taxa are uncertain.

In a molecular survey, Miller et al. (2010) find Homalonychidae are very closely related to Tengellidae, but the phylogenetic placement of both families was inconsistent. Penestomidae was very closely and consistently related to Zodariidae, with all four families included in the Zodariioidea clade. The possible relationship of *Homalonychus* with zodariioids opens the possibility of finding homologies in reproductive behavior; however, the sexual behavior of Tengellidae and Zodariidae is too slightly known (Barrantes 2008; Pekár & Král 2001; Pekár et al. 2005) to make comparisons and afford a basis for considering relationships with Homalonychidae.

However, a close phylogenetic relationship does not necessarily imply similarity of reproductive behavior, and the inferred gene trees do not necessarily correspond to species trees (Nichols 2001; Degnan & Rosenberg 2009). Hence, we suggest that courtship and mating behavior could be useful in reconstructing phylogenetic relationships in spiders, complementing morphological and molecular analyses, but with careful consideration of the possibility that similar behaviors could be cases of convergence. Studies of reproductive behavior and molecular analysis of zodariids and tengellids (including pisaurids) could help to reconstruct their phylogenetic relationships with homalonychids, as well as understand the evolution of reproductive behavior of all these little known spiders.

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Web decoration of *Micrathena sexpinosa* (Araneae: Araneidae): a frame-web-choice experiment with stingless bees

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Abstract. The function of silk web decorations in orb weaving spiders has been debated for decades. The most accepted hypothesized functions are that web decorations 1) provide camouflage against predators, 2) are an advertisement for vertebrates to avoid web damage, or 3) increase the attraction of prey to the web. Most studies have focused on only a few genera, *Argiope* being the most common. In this study, I evaluated the prey attraction hypothesis of silk decorations for a species of a poorly studied genus in this topic, *Micrathena sexpinosa* Hahn 1822. I used a web-choice experiment in which I presented empty or web-bearing frames at the end of a tunnel to stingless bees (*Tetragonisca angustula*). This frame-choice experiment consisted of the following comparisons: decorated web vs. empty frame, decorated web vs. undecorated web, and undecorated web vs. empty frame. Webs with decoration intercepted significantly more bees than empty frames and undecorated webs. Therefore, the decorations of *Micrathena sexpinosa* might play a role in increasing foraging success.

Keywords: Decorated, foraging, stabilimenta, undecorated

A diverse number of orb weaving spiders distributed in both tropical and temperate zones add silk web decorations, or stabilimenta, to their webs (Scharff & Coddington 1997). Their function is unknown, and at least six functions have been suggested for these structures (Herberstein et al. 2000; Bruce 2006). 1) They may camouflage the spider against predators (e.g., Eberhard 2003), 2) lure prey to the web (e.g., Li et al. 2004), 3) work as advertisement to vertebrates so as to avoid web damage (e.g., Eberhard 2006), 4) stabilize the web (Bruce 2006), 5) produce shade for thermoregulation of the spider (Humphreys 1992), or 6) collect water from the dew for the spider's consumption (Walter et al. 2008). The fact that web decorations are only found in diurnal species strongly suggests a visual function (Scharff & Coddington 1997). However, other possibilities are not necessarily mutually exclusive, although evidence supporting two or more functions at the same time for any species is lacking (but see Watanabe 1999, 2000).

Studies have mostly tested putative visual functions (Herberstein et al. 2000; Bruce 2006). Evidence in favor of the two most popular hypotheses (1 and 2) is contradictory. Several studies suggest that decorations can deter the attack of a predator or camouflage the spider (e.g., Blackledge & Wenzel 2001; Eberhard 2003; Li et al. 2003; Chou et al. 2005; Gonzaga & Vasconcellos-Neto 2005), but other researchers did not find evidence in favor of an anti-predator function (Herberstein 2000; Seah & Li 2001; Bruce et al. 2001; Li & Lim 2005; Eberhard 2006; Jaffé et al. 2006; Cheng & Tso 2007). One of the criticisms against this hypothesis is that decorations can attract predators to the web as well (e.g., Bruce et al. 2001).

In contrast, the prey-attraction function suggests that decorations could resemble UV gaps in vegetation, eliciting escape behavior in flying insects, or they could imitate food resources that reflect UV, luring prey (Craig & Bernard 1990). Many researchers found that decorated webs intercept more prey than undecorated webs (e.g., Watanabe 1999; Herberstein 2000; Bruce et al. 2001; Craig et al. 2001; Li et al. 2004; Li 2005; Bruce & Herberstein 2005; Cheng & Tso 2007), but some researchers found no evidence in favor of the hypothesis (e.g., Blackledge & Wenzel 1999; Hoese et al. 2006; Jaffé et al. 2006; Bush et al. 2008; Eberhard 2008; Gawryszewski & Motta 2008). One shortcoming of this hypothesis is that prey could apparently detect and avoid the web by the presence of the decoration (e.g., Blackledge & Wenzel 1999).

Using stingless bees, I tested the prey-attraction hypothesis for the less well-studied *Micrathena sexspinosa* Hahn 1822. *Micrathena* is a Neotropical genus that constructs web decorations (Herberstein et al. 2000). Nevertheless, no one has tested any hypothesis regarding the function of these decorations in any of the species. In contrast to the model genus *Argiope* with its polymorphism of designs (Herberstein 2000) that perhaps correlate to several functions (Bruce & Herberstein 2005), *M. sexspinosa* consistently produce the same decoration (D. Galvez pers. obs.), a line of silk on the top of the hub of the web (Fig. 1).

I used a trial tunnel in the field combined with decoration removal to test the preference of stingless bees for webs with decorations. In my design, prey nesting in a wooden box had to fly out of the tunnel and choose an exit in which the different web treatments were placed (Gálvez 2009). An advantage of this approach is that it mimics natural visual conditions better than laboratory experiments (Bruce 2006). I predicted that if web decorations function to attract prey, then decorated webs would intercept more bees than the undecorated webs or empty frames.

METHODS

Site & species.—I carried out these experiments at La Selva Biological Station in Heredia, Costa Rica (10°26′N, 83°59′W), a 1550-ha reserve in the Atlantic lowlands with an annual average rainfall of 4000 mm[3] (Sanford et al. 1994). *Micrathena sexspinosa* is a small orb-weaving spider occurring in the tropics that constructs its web in the midst of dense vegetation, woven on a vertical plane or slightly inclined (10–20°, Nenwtig

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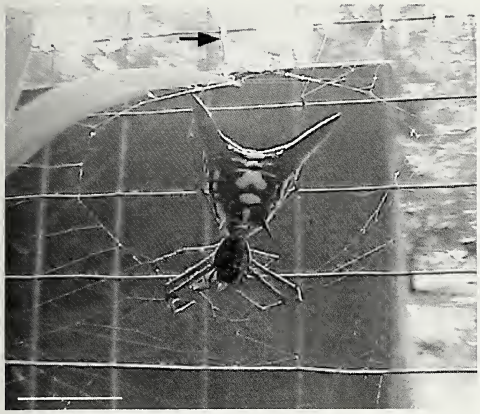

Figure 1.—Araneid *Micrathena sexspinosa* on its web eating a stingless bee. The spider rests at the center of the hole in the web; the decoration is built next to it. The arrow indicates part of the decoration. Scale bar = 1 cm.

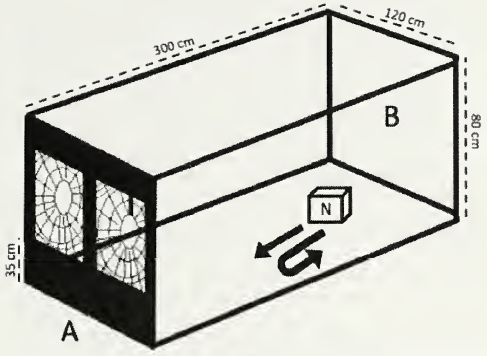

Figure 2.—Trial tunnel in which the *Tetragonisca angustula* stingless bees were exposed to the different web treatments of *Micrathena sexspinosa*. The walls and roof of the tunnel are not shown in order to reveal the interior. Both exits of the tunnel were opened (A and B); therefore bees could fly out of the tunnel from the nest (N) by either exit (arrows). See text for details about the frames bearing the webs. This figure depicts the comparison between a decorated (A right) and an undecorated web (A left).

1985), with a central hole through which the spider can move easily from one side to the other (Nentwig et al. 1993). Next to this hole, the spider usually builds a linear decoration like other *Micrathena* species (Herberstein et al. 2000). I identified the spiders using Levi (1985).

Experimental apparatus and treatments.—Without being systematic, I collected samples of *M. sexspinosa* and their webs daily from the field (around buildings and greenhouses) by sticking the webs to cardboard frames (18 × 18 cm), with a hole in the middle (324 cm^2). The side of the frame used to bear the web had adhesive tape placed with the sticky side facing the web. This tape was fixed to the frame by wrapping it to the corners of the frame with adhesive tape. I removed decorations from 16 out of 34 webs by burning the silk with a heated fine-pointed forceps while the spider was still on the web. In case some damage was done to the web during the burning process, particularly to the sticky spirals, I used the forceps to damage a similar area of the orb on the decorated web to be used for comparison. I collected a total of 34 spiders and used only one orb from each spider.

I placed the webs at the end of a 300 × 120 × 80 cm tunnel (Fig. 2), open at both exits, modified from Gálvez (2009). Since the frames did not match the area at the end of the tunnel, the remaining spaces were covered with cardboard. I placed a wooden box (40 × 30 × 20 cm) with a nest of the stingless bee *Tetragonisca angustula* Latreille 1811 at one of the ends of the tunnel. Thus the bees could fly out of the tunnel through either the end bearing the frames (A in Fig. 2) or the end next to the nest (B in Fig. 2); however, bees flew in or out always through the end bearing the frames (during the trials). I placed the nest in the tunnel with both exits opened for 48 h before the beginning of the experiments in order to get the bees acclimated to the tunnel and the new nest location.

I carried out a two-frame choice experiment in which the bees were exposed to two frames placed at the same end of the tunnel. Three variations of the choice experiment were performed: "decorated web vs. empty frame" (n = 8 pairs, 86 bees) "decorated web vs. undecorated web" (n = 9 pairs, 96 bees), and "undecorated web vs. empty frame" (n = 7 pairs, 72 bees). I kept the spiders on the webs and used individuals of similar sizes with the intention of comparing the two web treatments. I controlled the effect of web size, since the webs for each treatment always covered the same area in the frame (324 cm^2). The exit of the tunnel bearing the frames was in front of herbaceous vegetation, with a dark green mesh placed one meter from it in order to increase the contrast between the webs and the background (Bruce et al. 2005).

I counted the numbers of bees either being intercepted (including bees caught by spiders) or flying through the empty frame (hereafter referred to as "number of bees intercepted," although the empty frames could not intercept bees). I switched the relative (left/right) positions of the frames each time two bees had exited the tunnel or were intercepted in order to avoid any possible bias due to frame position. The frames were placed at the exit of the tunnel only when no bee was leaving the nest or flying in the tunnel. In cases in which three or more bees accumulated in the web because the spider did not attack them, I removed the frames and used forceps to remove the bees in order to avoid the possibility that bees caught there would deter more bees from flying into the web. The damage to the webs using this procedure was minimal and it was not taken into account for the analysis. I did not remove the bees if they were captured by the spider or wrapped with silk by the spider (1–2 bees per trial). After this, I put the frames back at the exit to continue the experiment. I used 9–10 bees per pair of frames, which required a new pair of webs made by fresh spiders.

I tested for a significant effect of web type on the likelihood of bee interception using a linear mixed model. I treated the

Table 1.—Statistical summary and preferences for the two-frame choice experiments set for *Micrathena sexpinosa*. Abbreviations: dec = decorated webs; undec = undecorated webs; empty = empty frames.

Treatment	Z	P	n	Total number of bees	% of bees intercepted		
					dec	empty	undec
dec vs. empty	3.90	< 0.001	8	86	65	35	----
undec vs. empty	0.829	0.407	7	72	----	46	54
dec vs. undec	2.74	0.006	9	95	60	----	40

counts of bees intercepted per web type in each trial as proportional data. I evaluated web type (between pair of frames) as the main effect and trial as random effect. Therefore, I carried out an analysis for each frame choice experiment. I accepted effects as statistically significant for $P \leq 0.05$, and I carried out all analyses in R 2.10.0 using the function lmer, specifying the binomial distribution for proportion data (R Development Core Team 2009).

RESULTS

In this two-frame choice experiment, I compared "decorated webs versus empty frames" for 8 pairs of frames (86 bees), "undecorated webs versus empty frames" for 7 pairs (72 bees) and 9 pairs (96 bees) for "decorated webs versus undecorated webs." Decorated webs intercepted significantly more bees (65%) than the empty frames (35%, $Z = 3.90$, $P < 0.001$, Table 1). Decorated webs intercepted more bees than undecorated webs as well (40%, $Z = 2.74$, $P = 0.006$, Table 1). I found no differences in the number of bees intercepted between undecorated webs and the empty frames ($Z = 0.829$, $P = 0.407$, Table 1).

DISCUSSION

The prey attraction hypothesis proposes that decorations may increase the foraging success of spider by luring prey to the web. *Micrathena sexspinosa* spiders on decorated webs intercepted significantly more bees than on empty frames and spiders on undecorated webs, which is in agreement with the hypothesis. The hypothesis has been partially supported among *Argiope* species; however, there is almost no support for other genera of araneids such as *Allocyclosa* (Eberhard 2003), *Araneus* (Eberhard 2008, but see Bruce et al. 2001), *Cyclosa* (Baba 2003; Chou et al. 2005; Gonzaga & Vasconcellos-Neto 2005, but see Tso 1998b) and *Gasteracantha* (Jaffé et al. 2006; Eberhard 2006; Gawryszewski & Motta 2008). The same can be said for the uloborids *Philoponella* (Eberhard 2006) and *Zosis* (formerly *Uloborus*, Bruce et al. 2005; Eberhard 2006).

There is a large variation of decorations at the species and individual level within these genera (Herbestein et al. 2000); in marked difference, *Micrathena* only shows a monophormic linear decoration pattern (Scharff & Coddington 1997). This varies from the polymorphism of decoration patterns found, for example, in the model genus *Argiope* that might be related to several functions (e.g., Bruce & Herberstein 2005). The linear pattern is probably primitive for the araneids *Argiope*, *Cyclosa* and *Gasteracantha* (Herberstein et al. 2000; Cheng et al. 2010). In contrast, it appeared de novo in *Micrathena* (Herberstein et al. 2000). Therefore, the function of web decorations in *Micrathena* might differ from its function in other genera. The lability of this trait, evolving at least nine times in 15 different genera, suggests the possibility of different functions (Scharff & Coddington 1997; Herberstein et al. 2000).

Multiple functions for decorations have almost no support in the literature, and *Micrathena sexspinosa*'s decoration does not seem to be an exception. For instance, individuals are found in confined spaces (e.g., shrubs) and therefore it is very unlikely that the decoration acts as a web advertisement for birds (e.g., Blackledge & Wenzel 1999; Jaffé et al. 2006; Eberhard 2006; Gawryszewski & Motta 2008). Furthermore, the decoration probably does not work as a mechanical barrier against predators, because the spider never rests behind the decorations, a behavior found in *Argiope* species (e.g., Li et al. 2003). Moreover, the size and shape of the decoration does not provide full cover to the spider. *Micrathena sexspinosa* generally builds its web in or between the vegetation; consequently, one side of the web is almost always unreachable to approaching predators (e.g., spider-hunting wasp). It seems that the main anti-predator response of *M. sexspinosa* is to shuttle to the other side of the web through the central hole in the hub or dropping from the web (pers. obs.).

Micrathena sexspinosa's decoration pattern does not appear to function for thermoregulation of the spider (Humphreys 1992). The decoration does not provide full shade against solar radiation, and the spider does not usually rest behind the decoration (pers. obs.). A mechanical function on the web also seems unlikely, since several individuals can be found near to each other on both decorated and undecorated webs under similar environmental conditions. If decorations were important for strengthening the web, then it is expected that spiders under similar environmental conditions would show similar decorating behaviors. However, I could not evaluate if an increase of the web tension occurs due to the decoration. For instance, *Octonoba sybotides* (Bösenberg & Strand 1906) build decorations that lure prey to the web (Watanabe 1999) and increase web tension (Watanabe 2000), which allows the spider to respond faster to small prey caught in the web. Therefore, these two functions are not necessarily mutually exclusive, and both increase foraging success of the spider.

Luring prey to the web might not depend entirely on the web decoration but perhaps on the spider coloration as well (e.g., *Argiope* spp., Craig & Ebert 1994; Tso et al. 2002; Cheng & Tso 2007; Bush et al. 2008). The lack of significant differences between undecorated webs and empty frames does not support the prey-attraction function of body coloration as suggested for other araneids. However, this study was not designed to evaluate the effect of spider morphology on prey behavior. In *Micrathena gracilis* (Walckenaer 1805), Vanderhoff et al. (2008) did not find any effect of spider presence on prey capture rate, nor did he find differences between control and black-painted spiders. Therefore, body coloration of *M.*

sexspinosa might serve for another function, for instance in camouflage of the spider (Hoese et al. 2006; Václav & Prokop 2006). Nevertheless, the best method for evaluating the effect of the spider (e.g., coloration) on prey attraction is by comparing webs with spiders against webs without spiders, a comparison I did not include in this study. In addition, the spectral measurements of the decorations, spiders and the background can be used to evaluate their visibility to prey in order to confirm the prey attraction function (e.g., Bruce et al. 2005).

The decorating behavior of *M. sexpinosa* could offer a great advantage for resource use; however, further research is needed in order to evaluate whether a disadvantage of building the decoration exists as in other decorating species (Bruce 2006, Herberstein et al. 2000).

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Trophic strategy of ant-eating *Mexcala elegans* (Araneae: Salticidae): looking for evidence of evolution of prey-specialization

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Abstract. We investigated the trophic strategy of *Mexcala elegans* Peckham & Peckham 1903, an ant-eating salticid spider from South Africa, in order to gain baseline information concerning the evolution of prey specialization. We studied its natural prey, prey acceptance, and choice using a variety of prey species. In its natural habitat, the spider captured only ants, mainly its mimetic model *Camponotus cinctellus*, indicating that the species is a stenophagous ant-eater. However, in the laboratory, *M. elegans* captured 12 different invertebrate taxa with efficiency similar to the capture of ants, suggesting that it is euryphagous. For the capture of ants but not for other prey, it used a specialized prey-capture behavior. In prey-choice experiments, the spiders did not prefer ants to flies. We found no evidence for neural and behavioral constraints related to identification and handling of prey. Our results suggest that *M. elegans* is a euryphagous specialist using a specialized ant-eating capture strategy in which prey specialization has evolved as a byproduct of risk aversion ("enemy-free space" hypothesis).

Keywords: Prey, hunting behavior, myrmecophagy, mimicry, evolution

Stenophagy, the utilization of a narrow prey range, may be a product of an innate response due to evolutionary transitions and fitness trade-offs or a proximate response due to specific environmental conditions; i.e., dominance of a certain prey species. In the former case, such species are stenophagous specialists because they are not able to catch and utilize alternative prey. In the latter case, such predators are stenophagous generalists since they possess versatile adaptations allowing them to capture and process a variety of prey in environments with diverse prey (Sherry 1990).

Evolution of stenophagous specialists has been explained by a number of hypotheses (particularly in herbivores). The enemy-free space hypothesis postulates that stenophagy has evolved as a byproduct of using host/prey as a refuge or defense (Brower 1958). The neural constraints hypothesis (Jermy et al. 1990) suggests an inability to recognize cues from other than preferred prey. The physiological trade-off hypothesis (Singer 2001) is relevant when the predator is constrained in utilization of other than its preferred food. And, the optimal-foraging hypothesis (Singer 2008) predicts lower efficacy in the capture of alternative prey.

Revealing the trophic strategy of a species requires multiple approaches. Analysis of natural prey alone cannot provide complete evidence for a trophic strategy. Such data need to be supplemented by extensive laboratory prey acceptance and choice experiments. This is because the natural prey analysis reveals only the realized trophic niche that measures actual diet use and results from the effect of both intrinsic and extrinsic variables. In contrast, laboratory experiments can reveal the fundamental trophic niche that is determined by intrinsic variables only (Bolnick et al. 2003). Furthermore, trade-offs (behavioral, morphological, or physiological) that constrain prey utilization in stenophagous specialists can only be determined experimentally. The gathered evidence can then be used to draw conclusions on the trophic strategy.

Spiders have been found to be mainly euryphagous (Nentwig 1987), but there are quite a few cases of stenophagous species. Evidence for stenophagy is mainly anecdotal. The most frequent type of stenophagy observed is myrmecophagy; spiders in several families (e.g., Zodariidae, Gnaphosidae, Theridiidae) demonstrate specialization in ant predation (Heller 1976; Carico 1978; Pekár 2004). While the majority of salticid spiders rarely feeds on ants (e.g., Nentwig 1986; Guseinov 2004), some tropical species are myrmecophagous (Cutler 1980; Wing 1983; Jackson & Van Olphen 1992; Li et al. 1999; Allan & Elgar 2001; Jackson & Li 2001). These myrmecophagous species use a specialized tactic to capture ants (e.g., Jackson & Van Olphen 1992; Jackson & Li 2001). However, no salticid species is known to prey exclusively on ants.

We investigated the prey capture behavior of a salticid spider *Mexcala elegans* Peckham & Peckham 1903 in South Africa. *Mexcala elegans* appears to be an inaccurate Batesian mimic of a few ground-living ant species. It is a distinctively polymorphic spider, with three color variations: 1) a metallic silver-gray body with black triangular abdominal marking in late instar immature and adult specimens, resembling silver-gray ground-dwelling ants (Fig. 1A), presumably *Camponotus cinctellus* that are common on the ground surface and low foliage in northeastern South Africa; 2) a metallic silver-gray body adorned by two pairs of large yellow abdominal spots (Fig. 1B) in adult specimens resembling large ground-dwelling wingless female mutillid wasps; and 3) a metallic blue prosoma and bright metallic green abdomen in early instar immatures, possibly inaccurate ant mimics.

Other species of the genus *Mexcala* feed on their ant models (Curtis 1988). Therefore, we predicted that *M. elegans* also hunts its model ants, thus supporting the enemy-free space hypothesis. In order to reveal any trade-offs, neural or behavioral, that would lead to support alternative evolutionary hypotheses, we performed both field and laboratory

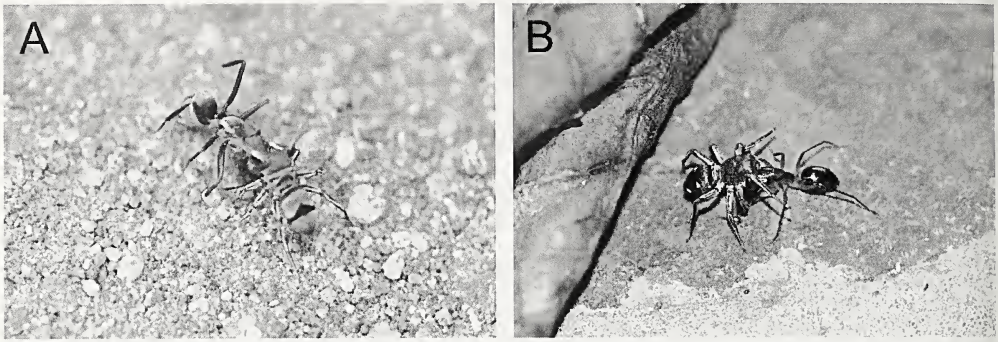

Figure 1.—*Mexcala elegans* capturing ants in the field. A. Female of the gray color variation capturing *Camponotus cinctellus*; B. Female of the spotted variation capturing *Camponotus* sp. 2.

surveys. After examining natural prey capture in the field, we tested the ability of this species to catch and eat alternative prey in the laboratory, and also whether it prefers ants to alternative prey.

METHODS

Field survey.—We investigated the natural prey of *M. elegans* during field trips to Ndumo Game Reserve, South Africa in June–July and November–December 2004–2009 (11 trips in total) that formed part of a larger arachnid biodiversity survey in the reserve. We collected 64 *M. elegans* spiders in a variety of habitats: *Acacia nigrescens* woodland (1.6% of total), *A. xanthophloea* forest (7.8%), broadleaf woodland (25%), floodplains (25%), *Ficus sycomorus* forest (3.1%), and subtropical bush (37.5%). Individual spiders were followed for up to 10 minutes to see whether they would capture ants and to note the prey capture behavior and interactions with different ant species. If they had a prey in their chelicerae, the spiders were collected and preserved in ethanol and brought to laboratory where their sex and the prey was identified to species level. We measured the size of adult males (n = 15) and females (n = 15) and 15 ant workers of each species captured in the field using an ocular micrometer within a binocular stereomicroscope.

Laboratory experiments.—For intensive studies of prey capture and prey choice, we brought 15 live juvenile *M. elegans* (body size 3.5–5.3mm) collected at Ndumo Game Reserve to the home laboratory. We housed spiders individually in Petri dishes (diam. 4.5 cm) with a filter paper attached to the bottom. A small piece of cotton moistened at 2-day intervals served as a water resource. Using these spiders, we performed two different experiments.

In the acceptance experiment, we used a complete repeated measures design, offering each spider (n = 15) each of 17 potential prey species in random order (Table 2). The prey were not native to the spider, as the experiments were performed in Europe, but we used only prey from orders that also occur in South Africa. The relative body size of the prey (1.6–8.0 mm) to spider body length (3.3–5.3 mm) was 0.3–2.4. We observed each trial continuously. If spiders did not respond to a prey item within 15 min, we stopped the trial and 12 h later initiated a new trial with a different prey. If a prey was accepted, we initiated the next trial 24 h later. For each trial, we recorded whether the prey was attacked and subsequently consumed. In trials with ant or termite prey, we also recorded the latency to attack (i.e., time between the spider orientation toward the prey and the attack) and the latency to paralysis (i.e., time between the attack and grabbing the prey in the chelicerae).

In the prey-choice experiment, performed after the acceptance experiment with a paired design, we released two non-native prey items of similar size (relative prey/spider size: 0.4–1) at the same time into the dish occupied by a spider. Spiders (n = 15) were starved for two days prior to each trial. We used an ant, *Tetramorium caespitum* (Myrmicinae), and a fly, *Drosophila melanogaster* (Drosophilidae), or two ant species, *T. caespitum* and *Lasius niger* (Formicinae). These two alternative treatments were repeated for each individual on a random basis. In these paired trials, we recorded which of the two prey insects was attacked and which one was consumed. At least one of the prey insects was attacked and consumed in each trial. All experiments were performed between 09:00 and 16:00 h.

Data analysis.—We analyzed data using various methods within R (R Core Development Team 2009). For the field data, we used ANOVA to compare prey size among immature, adult male and adult female spiders. Because there were repeated measures of the same individuals in both experiments, we used Generalized Estimating Equations (GEE) as an alternative to Generalized Linear Models. This method allows implementation of an association (correlation) structure that corrects for too small standard errors of parameter estimates and inferences favoring acceptance of the alternative hypothesis (Hardin & Hilbe 2003). We used GEE with binomial error structure (GEE-b) to compare capture frequency of the prey acceptance experiment, since the response variables were relative frequencies. We used GEE with Gamma errors and log link (GEE-g) to compare latencies among selected prey species, as the response variable was time, and variance was expected to increase with the mean. We used a proportion test to compare the frequency of attack and consumption separately for selected prey species. We analyzed the prey-choice experiments data with the McNemar test due to paired trials.

Table 1.—Natural prey of juvenile, male, and female *Mexcala elegans* specimens determined during field observations in Ndumo Game Reserve from 2004 to 2009. The size is an average total body length of workers attacked by spiders.

Ants		Spider predators			
Subfamily/species	Size [mm]	Juveniles	Males	Females	Total
Formicinae					
Anoplolepis custodiens (Smith)	5.9	0	1	4	5
Camponotus cinctellus (Gerstäcker)	7.2	6	12	6	24
Camponotus sp. 2 (*maculatus* group)	8.6	2	3	3	8
Polyrhachis sp.	8.6	0	4	5	9
Myrmicinae					
Crematogaster sp.	3.5	2	0	1	3
Myrmicaria natalensis (Smith)	6.3	0	1	3	4
Tetramorium quadrispinosum Emery	3.5	3	0	0	3
Ponerinae					
Pachycondyla tarsata (Fabricius)	16.5	0	0	4	4
Streblognathus peetersi Robertson	11.6	0	0	2	2
Pseudomyrmicinae					
Tetraponera ambigua (Emery)	6.8	2	0	0	2
Total		15	21	28	64

RESULTS

Field survey.—In the field, *M. elegans* captured and consumed ten species of ants from four subfamilies (Table 1). We observed no prey other than ants being captured. Among ants, the most frequent prey was *Camponotus cinctellus*. Adult male (body size 5.3–8.3 mm) and female (6.1–8.9 mm, Fig. 1) *M. elegans* captured significantly larger ant species (*Camponotus*, *Polyrhachis*, *Anoplolepis* and *Myrmicaria*) than the juveniles, which generally preyed on smaller ants such as *Crematogaster*, *Tetramorium*, and *Tetraponera* (ANOVA, $F_{2,60} = 4.5$, $P = 0.013$, Fig. 2).

Laboratory experiments.—Although the prey acceptance experiment showed that the spiders were capable of attacking diverse prey, and the prey choice experiment showed no preference between prey types, the spiders did respond differently to varying prey types. In the acceptance experiment, spiders responded differently to the 17 potential prey species. The frequency of attacks differed among the 17 prey species (GEE-b, $X^2_{16} = 194$, $P < 0.0001$). Spiders did not attack crickets, beetles, *Theridion* spiders, or woodlice and springtails and beetle larvae were only attacked by half of the spiders. Other prey species such as ants, *Pardosa* spiders, termites, flies, and moths were always attacked (Table 2). Although spiders consumed the majority of prey species they attacked, they were less likely to consume *Tribolium* larvae and *Pardosa* spiders (Proportion tests, $X^2_1 > 5.5$, $P < 0.02$). Spiders attacked prey that were on average 1.03 of their body length ($Q_{25} = 0.64$, $Q_{75} = 2.2$, $n = 255$). In the choice experiments, spiders attacked and consumed ants as frequently as flies (McNemar tests, $X^2_1 = 0$, $P = 1$ $n = 15$). Similarly, spiders attacked and consumed *Lasius* ants as frequently as *Tetramorium* ants (McNemar tests, $X^2_1 > 0.4$, $P > 0.5$).

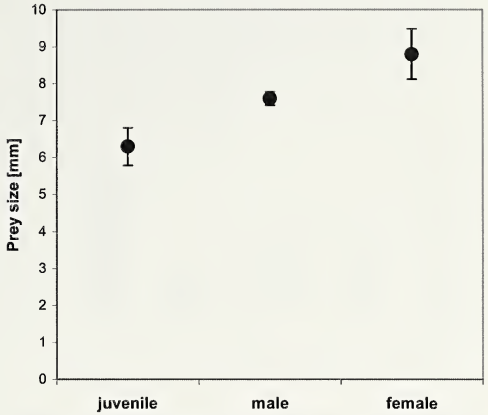

Figure 2.—Comparison of the prey size (mean ± SE) captured by juveniles, males and females in the field.

Mexcala elegans used different predatory behavior to catch different prey taxa. Although spiders ignored woodlice and beetles, they stalked aphids, crickets, bugs, and *Theridion* spiders but did not attack them. Spiders grabbed small springtails, leafhoppers, moths, and flies with their forelegs and moved them to their chelicerae. In contrast, they repeatedly attacked termites head-on, and then grabbed hold of the insect's thorax. To catch ants, the spider approached from the rear, maintaining a distance of three to four body lengths from an ant, all the while moving the front legs and abdomen up and down. The spider attacked quickly from behind, biting the ant on the abdomen. The spider then retreated and followed its ailing prey with raised forelegs (Fig. 3A), maintaining a distance of about two body lengths. Once the ant slowed down, the spider grabbed the ant's antenna with its chelicerae (Fig. 3B), and after a minute, it moved its hold to the thorax.

Among the four ant and one termite species used in the trials, the spiders showed significantly different latency in their attacks (GEE-g, $X^2_4 = 9.6$, $P = 0.047$, Fig. 4A). Spiders attacked *Lasius* and *Messor* ants with a significantly shorter latency than *Formica* ants (contrasts, $P < 0.02$). There was also a significantly different paralysis latency among these prey ants (GEE-g, $X^2_4 = 49.4$, $P < 0.0001$, Fig. 4B). Large *Formica* and *Messor* ants had a significantly longer latency to paralysis than small *Lasius* and *Tetramorium* ants (contrasts, $P < 0.03$). Termites of the same size as small ants were paralyzed more quickly than all ant species (contrasts, $P < 0.0001$).

Table 2.—List of prey used in laboratory experiment. The size of prey is an average total body length. $n = 15$ trials for each species. Percentage of consumed is of those that were attacked.

Order/species	Size [mm]	% Attacked	% Consumed
Araneae			
Theridion sp.	3.0	0	0
Pardosa sp.	2.5	100	10
Isopoda			
Porcellio scaber Latreille	3.5	0	0
Collembola			
Sinella curviseta Brook	1.6	45.5	100
Isoptera			
Reticulitermes sp.	4.7	100	100
Ensifera			
Acheta domesticus (Linnaeus)	3.5	0	0
Heteroptera			
Lygus pratensis (Linnaeus)	6.0	0	0
Sternorhyncha			
Aphis fabae Scopoli	1.7	9.1	0
Auchenorhyncha			
Eupteryx sp.	3.5	81.8	100
Lepidoptera			
Plodia interpunctella (Hubner)	6.5	81.8	100
Hymenoptera			
Formica pratensis Retzius	6.3	100	100
Lasius niger (Linnaeus)	3.5	100	100
Messor muticus (Nylander)	6.0	91.7	100
Tetramorium caespitum (Linnaeus)	3.5	91.7	100
Coleoptera			
Phylotreta sp. imago	3.3	0	0
Tribolium castaneum (Herbst) larva	8.0	50	0
Diptera			
Drosophila melanogaster Meigen	2.0	100	100

DISCUSSION

We found a contrasting trophic strategy in *M. elegans*. Our field observations suggest a stenophagous habit, but laboratory experiments conversely indicate a euryphagous habit. In the field, *M. elegans* captured only ants. This is consistent with observations of two other species of this genus, *M. namibica* Wesołowska 2009 and *M. rufa* Peckham & Peckham 1902 from Namibia, that feed on *Camponotus fulvopilosus* (Curtis 1988). In the laboratory, however, *M. elegans* caught a wide variety of prey. So, the fundamental trophic niche includes a wide assortment of prey, whereas the realized niche includes only ants.

Mexcala elegans recognized and captured prey other than ants as efficiently, or even more efficiently, than ants. Thus neural and behavioral trade-offs resulting in an inability to recognize cues from other prey and to catch non-ant prey were not present. This is in contrast to stenophagous ant-eaters of the genus *Zodarion*, for example, which are unable to subdue prey other than ants (Pekár 2004; Pekár & Toft 2009). Yet *M. elegans* used completely different behavior to catch ants than other prey, so this species has clearly evolved a specialized capture strategy that seems to be very effective and safe for ant capture, as we have not witnessed a single successful reversed attack by an ant toward the spiders in laboratory experiments (0%, $n = 60$, pooled across the acceptance trials with ants).

Mexcala elegans used a 'bite-and-release' tactic to catch ants. This specific tactic is also used by other ant-eating salticids, namely *Naphrys pulex* (Hentz 1846), *Aelurillus muganicus* Dunin 1984, and *Tutelina similis* (Banks 1895) (Wing 1983; Li et al. 1996; Huseynov et al. 2005). This special tactic includes a short leap with a quick bite, followed by release and retreat. Interestingly, a similar tactic is used by other non-salticid, ant-eating spiders, such as gnaphosids, zodariids, and thomisids (Heller 1976; Lubin 1983; Oliveira & Sazima 1985; Pekár 2004). In all cases, the spiders usually attack either head-on; i.e., bites between head and thorax (Edwards et al. 1974), or from the rear; i.e., on the abdomen or legs (Jackson & Van Olphen 1992; Jackson et al. 1998), both tactics making it impossible for the ant to defend itself.

As the most frequent natural prey of *M. elegans* were *Camponotus* ants (subfamily Formicinae), we expected that

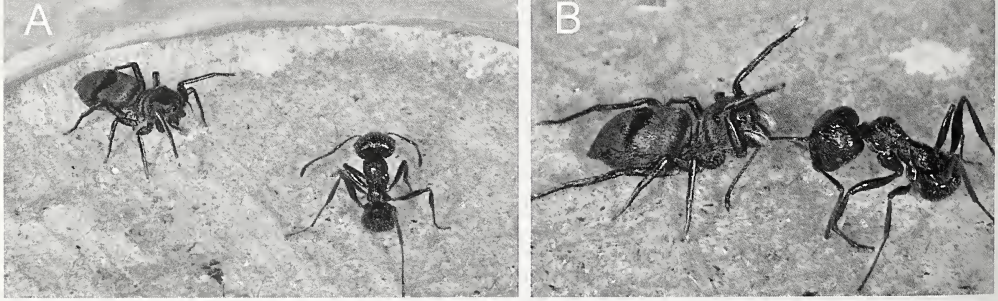

Figure 3.—Predatory behavior of *M. elegans* when capturing ants. A. Spider stalks attacked ant with raised forelegs. B. Spider grabs antennae of ant in chelicerae.

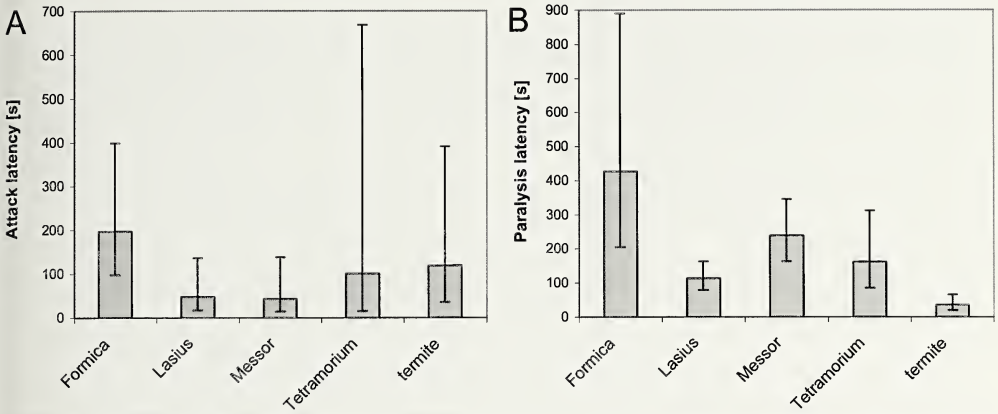

Figure 4.—Comparison of the attack latency (A) and paralysis latency (B) for four ant (*Formica*, *Lasius*, *Messor*, *Tetramorium*) and one termite species. Bars indicate means, whiskers indicate 95% confidence intervals of each mean.

related ants (*Formica* and *Lasius*) would be attacked and paralyzed more quickly than others. The spiders attacked four ant species used in the acceptance trials at significantly different latencies. Slow-moving species (*Messor* and *Lasius*) were attacked more rapidly than fast-moving *Formica*. Larger ant species had longer paralysis latencies than small ant species, regardless of their taxonomic relatedness, suggesting that the venom of *M. elegans* is not specific for certain subfamilies of ants, as was found in ant-eating *Zodarion* (Pekár et al. 2008).

In the field, *Mexcala elegans* frequently captures ants with a greater body length than itself; the largest, *Pachycondyla tarsata*, is double the spider's body length. Similarly, in laboratory experiments, the spiders captured prey up to twice their own length, consistent with observations of other myrmecophagous spiders that catch prey much larger than themselves (e.g., Soyer 1943; Pekár 2004).

Absence of neural and behavioral trade-offs does not preclude the presence of physiological trade-offs. We have not studied the effect of prey type on fitness aspects such as survival or reproduction. Thus we cannot exclude the possibility that *M. elegans* has evolved a physiological trade-off in their utilization of alternative prey. However, in another ant-eating salticid, *Siler cupreus* (Simon 1889), Miyashita (1991) did not find evidence for either behavioral or physiological trade-offs, as the spider was able to catch alternative prey and suffered high mortality when reared on a pure ant diet. Therefore, we expect that physiological trade-offs may not have evolved in *M. elegans*, either. If our predictions are correct, then the evolution of stenophagy in *M. elegans* cannot be explained by the physiological trade-off hypothesis.

Mexcala elegans, like *M. rufa* and *M. namibica*, not only imitates ants but also feeds on the model species (Curtis 1988). It is therefore likely a Batesian mimic. This spider associates closely with its ant models, which are abundant in a variety of habitats. Myrmecomorphy, combined with spatial association with ants, may provide *M. elegans* with higher protection from enemies. Thus it appears to favor the enemy-free space hypothesis.

We conclude that the evidence gained on the trophic strategy of *M. elegans* suggests that it is a euryphagous specialist, because it has the versatility to catch a variety of prey but uses a specialized prey capture tactic on ants. Observed stenophagy in the field has presumably resulted as a byproduct of adaptive dynamics related to risk aversion (avoiding of enemies).

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Determinants of differential reproductive allocation in wolf and nursery-web spiders

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Abstract. We used data from 33 species of cursorial spiders in northern Mississippi (USA) to investigate the relative contributions of ecology and phylogeny to the reproductive trade-off between number and size of offspring. Sixty percent of the variation among genera for female reproductive allocation was due to differences between the family Pisauridae and the family Lycosidae. Temporal variation in reproductive allocation during the reproductive season was not observed for the majority of species examined. We found significantly different patterns of reproductive allocation among species within genera, suggesting that each species has responded to distinct selection pressures. Preliminarily, this extensive variation appears to be due mostly to interspecific competition and predation risk from other spiders. However, the patterns of reproductive allocation of species within a single guild (i.e., a group of species potentially competing for the same resources) for the two families are very different. Larger species of wolf spiders (family Lycosidae) within a given guild produce smaller numbers of larger offspring relative to the size of the mother, and smaller species produce the reverse. However, in nursery-web spiders (family Pisauridae) the larger species within a guild produce larger numbers of smaller offspring than expected. The current study provides an example of the flexibility of life history evolution despite phylogenetic constraints. It also demonstrates the potential for varying life history strategies to mediate competition, allowing similar species to coexist.

Keywords: Fecundity, interspecific competition, life-history evolution, Lycosoidea, Pisauridae, predatory dominance, trade-offs

Life history theory predicts a trade-off between the number of offspring produced and the size of those offspring, given the finite amount of resources available to individuals (Stearns 1992; Roff 2002). Females can invest in producing either a larger number of smaller offspring or fewer larger offspring. The observed pattern of maternal resource allocation (few large or many small) may result from environmental influences and/or phylogenetic constraints (Marshall & Gittleman 1994), with natural selection acting to produce a clutch size that maximizes the genetic contribution to the next generation within those constraints (Lack 1947; Stearns 1992; Fox & Czesak 2000). Differences in the way females allocate maternal resources should reflect selective pressures (mortality regimes) specific to the biotic and abiotic environment (Fox & Czesak 2000).

Pisauridae (nursery-web spiders) and Lycosidae (wolf spiders) are closely related families in the superfamily Lycosoidea (Coddington 2005). Species within each family exhibit qualities that make them ideal for testing hypotheses concerning the evolution of the allocation of reproductive resources. First, females exhibit similar but not identical levels of parental care, and offspring of the two families may face differential predation risk due to the mode of maternal care. Maternal care in both families can be divided into pre- and post-emergence stages. During the pre-emergence stage, wolf spider females carry egg sacs suspended from their spinnerets, and nursery-web females carry egg sacs in their chelicerae. The post-emergence stage begins after a period of 4–6 wk for wolf spiders and 2–3 wk for nursery-web spiders (this study), when females must tear open the egg sac in order for spiderlings to emerge. In wolf spiders, once the egg sac has been opened the spiderlings emerge and crawl onto their mother's abdomen where they remain for 1–2 wk before dispersing. Nursery-web females, on the other hand, suspend the opened egg sac from a specially constructed 3-dimensional web structure. Emerging spiderlings crawl onto the nursery web and remain there approximately 1–2 wk before dispersing. During this period, the female does not abandon her offspring but remains close by, presumably to defend her young (but see Kreiter & Wise 2001).

Second, the populations we used of these species are semelparous. Inclusion of iteroparous species can introduce confounding effects of trade-offs between current and future reproduction and current reproduction and future survival (e.g., Desouhant et al. 2005; Waelti & Reyer 2007).

Third, species of both families are found in a variety of habitats and are almost exclusively cursorial hunters. Thus, the possibility for extensive adaptation to specific habitats exists as well as the potential for strong competition among species in the same habitats.

In wolf (Araneae: Lycosidae) and nursery-web (Araneae: Pisauridae) spiders in Mississippi, we have shown that a trade-off does exist between size and number of offspring, and that there is no significant variation among species in the proportion of available resources allocated to total reproductive effort (Nicholas et al. 2011). In the current paper, our primary question is: Given the trade-off presented in Nicholas et al. (2011), how do phylogeny, interspecific competition, and temporal heterogeneity in the timing of reproduction interact to determine among-species patterns of maternal resources partitioning between number and size of offspring? Specific hypotheses are: 1) Do species or genera that are more closely evolutionarily related share more similar patterns of reproductive resource allocation? 2) Do potentially competing

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species within a guild show consistent patterns of reproductive allocation of resources among guilds? 3) Do individual species shift reproductive resource allocation during the reproductive season?

METHODS

We housed spiders and calculated reproductive output as in Nicholas et al.(2011). Briefly, we used wild-caught females representing 28 morphospecies of wolf spiders from ten genera and five species of nursery-web spiders from two genera. Sample sizes for individual morphospecies can be found in Table 1 of Nicholas et al. (2011).

Measuring fecundity.—We opportunistically collected females with egg sacs throughout Mississippi from March–September 2004–2006. Some gravid females were also captured, but individuals not producing an egg sac within 48 h were not used for the study to avoid the confounding effects of supplemental laboratory feeding. Most of the species included in this study are nocturnal, and we collected at night using a headlamp to locate eye shine. Several of the wolf spider species have not been previously described and we classified them as morphospecies. All together, we collected 28 morphospecies of wolf spiders belonging to the following genera (with number of species in that genus in paretheses): *Allocosa* (1), *Geolycosa* (2), *Gladicosa* (1), *Hogna* (7), *Pardosa* (3), *Pirata* (2), *Rabidosa* (4), *Schizocosa* (6), *Trochosa* (1), and *Varacosa* (1) and five species of nursery-web spiders within the genera *Dolomedes* (3) and *Pisaurina* (2). We deposited voucher specimens in the Mississippi Entomological Museum. The number of individuals per species collected was highly variable, with a mean of 27.7 and a median of five (Nicholas et al. 2011).

We brought females into the laboratory and maintained them individually in plastic containers measuring 22 cm by 15 cm. The containers were filled with several cm of commercial topsoil, and dried grass stems were added to provide places for spiders to perch. We kept larger individuals of Pisauridae in 38-l aquariums filled with several cm of commercial topsoil and 2–3 large sheets of pine tree bark provided as a substrate for nursery web construction. We misted containers every other day to provide moisture. In our experience (Nicholas et al. 2011), females carrying egg sacs did not feed, so that laboratory diet is not a confounding factor on fecundity or resource allocation. Any burrowing behavior, date of egg sac construction, and date of hatching were recorded at each misting or feeding.

We made the following observations for all wolf spiders. When all spiderlings emerged, we weighed the female and her spiderlings to the nearest milligram. The female was then anesthetized with CO_2 gas and the spiderlings were removed using a soft paint brush. We then weighed the female without the spiderlings, and $\geq$ 30 spiderlings were counted and weighed en masse. We collected similar data from nursery-web spiders except that we did not need to anesthetize females or spiderlings because they are living on a nursery web, eliminating the need for anesthetization to remove offspring. For species producing fewer than 100 spiderlings, all offspring were counted directly. We estimated mean spiderling mass, number of offspring (in species with > 100 spiderlings/clutch), and total clutch mass using the following equations:

$$\text{Total clutch mass} = \text{Mass (Female + spiderlings)} - \text{Mass (Female alone)}$$

$$\text{Mean spiderling mass} = \text{Total mass of spiderlings counted} / \text{Number of spiderlings counted}$$

$$\text{Total number of offspring} = \text{Total clutch mass} / \text{Mean spiderling mass}$$

Ecological community.—We used "ecological community" to identify potentially competing suites of species. Ecological community contains a spatial component (habitat type) and a temporal component (timing of offspring hatching: time of hatching is important because similarly-sized individuals are more likely to compete). We classified habitat type as forest (pine, deciduous, or mixed stands of trees) or grassland. We distinguished three seasons of offspring hatch: spring, summer, or fall. Thus, ecological community describes a guild of spiders that is born in the same season and use the same habitat.

Data analyses.—Contribution of phylogeny. We test the hypothesis that phylogenetic relations influence the patterns of reproductive allocation of resources in the families Lycosidae and Pisauridae. Increasingly, researchers have used comparative methods to examine various patterns of life history traits across species. However, traits measured from related groups may not be independent data points, and phylogenetic relationships should be considered in any comparative study (Freckleton et al. 2002; Blomberg et al. 2003; Desdevises et al. 2003). When not taken into account, phylogenetic autocorrelation can lead to erroneous conclusions concerning the evolution of traits under consideration (Blomberg et al. 2003). As suggested by Stearns (1992), we examined the amount of variance in reproductive allocation at different taxonomic levels using a nested analysis of variance. The taxonomic level explaining the majority of variation in a life history trait provides the most independent level of comparison and reduces the confounding effect of phylogenetic relationships, and thus is the level at which further analyses should be conducted. We conducted a nested analysis of variance with the independent variables of species within genera, genera within family, and family. The independent variable, reproductive allocation, was derived from a principal components analysis of female mass, offspring mass, and number of offspring. This allowed us to identify the components that explicitly describe the trade-off between offspring mass and offspring number (i.e., reproductive allocation) (see Nicholas et al. 2011).

To test whether species within a genus differed significantly in reproductive allocation, we examined separately the three wolf spider genera for which we had data on more than three species (*Hogna*, *Rabidosa*, and *Schizocosa*). Residual offspring mass and number were derived from a least squares linear regression between log female mass and log offspring mass and between female mass and number of offspring. We conducted a separate analysis of variance for each genus, with species as the independent variable and residual offspring mass and residual number of offspring as dependent variables.

Table 1.—Summary of some life history data for species collected. The tabled information includes means and standard errors for: mass of females in mg (Maternal), the mean number of offspring produced per clutch (Fecundity), mean spiderling mass in mg (Offspring mass); as well as classification of ecological community. Species were designated as: 1) hatching in the spring (Sp), summer (Su), or fall (Fl); and 2) found in forest (F) or grassland (G) habitats. Their spatial and temporal separation divided them into ecological communities.

Species	Maternal mass	Fecundity	Offspring mass	Ecological community
Lycosidae				
Allocosa funereal (Hentz 1844)	17	56	0.24	SuG
Geolycosa fatifera (Kurata 1939)	542	118	1.50	SuG
Geolycosa missouriensis (Banks 1895)	742 ± 21	133 ± 18	1.83 ± 0.01	SuG
Gladicosa pulcra (Keyserling 1877)	301 ± 19	164 ± 28	1.13 ± 0.03	SpF
Hogna annexa (Chamberlin & Ivie 1944)	246 ± 13	219 ± 20	0.72 ± 0.02	SuG
Hogna aspersa (Hentz 1844)	1288 ± 125	268 ± 68	2.59 ± 0.08	SuF
Hogna georgicola (Walckenaer 1837)	840 ± 39	236 ± 15	2.19 ± 0.03	SuF
Hogna lenta A	599 ± 37	206 ± 13	2.03 ± 0.07	SuG
Hogna lenta B	642 ± 53	569 ± 61	0.70 ± 0.03	FlG
Hogna wallacei (Chamberlin & Ivie 1944)	544 ± 63	228 ± 45	1.19 ± 0.03	SuG
Hogna watsoni (Gertsch 1934)	140	60	1.01	SuG
Pardosa cocinna (Thorell 1877)	35 ± 2	60 ± 12	0.36 ± 0.01	SuG
Pardosa milvina (Hentz 1844)	20 ± 5	40 ± 3	0.47 ± 0.01	SpG
Pardosa pauxilla (Montgomery 1904)	12	18	0.33	SuF
Pirata species A	12 ± 1	28 ± 3	0.37 ± 0.01	SuG
Pirata species B	35	74	0.24	SuF
Rabidosa carrana (Bryant 1934)	592 ± 145	187 ± 93	1.83 ± 0.19	SpG
Rabidosa hentzi (Banks 1904)	250 ± 33	90 ± 30	1.66 ± 0.17	SuF
Rabidosa punctulata (Hentz 1844)	415 ± 5	143 ± 3	1.36 ± 0.01	SpG
Rabidosa rabida (Walckenaer 1837)	599 ± 12	356 ± 9	1.05 ± 0.01	SuG
Schizocosa avida (Walckenaer 1837)	241 ± 16	212 ± 22	0.50 ± 0.02	SuG
Schizocosa bilineata (Emerton 1885)	66 ± 44	28 ± 5	0.47 ± 0.03	SuG
Schizocosa duplex (Chamberlin 1925)	67 ± 7	76 ± 15	0.57 ± 0.02	SuF
Schizocosa ocreata gr.	70 ± 5	80 ± 7	0.60 ± 0.01	SuF
Schizocosa saltatrix (Hentz 1844)	102 ± 11	116 ± 9	0.65 ± 0.01	SP
Schizocosa uetzi (Stratton 1997)	73	63	0.58	SuF
Trochosa acompa (Montgomery 1902)	88 ± 11	102 ± 13	0.70 ± 0.01	SuG
Varacosa avara (Keyserling 1877)	96 ± 28	73 ± 12	0.95 ± 0.07	SpG
Pisauridae				
Dolomedes albineus (Latreille 1804)	736 ± 129	668 ± 58	0.97 ± 0.02	SuF
Dolomedes tenebrosus (Hentz 1844)	1947	2627	0.59	SuF
Dolomedes triton (Walckenaer 1837)	642 ± 32	1147 ± 530	0.44 ± 0.00	SuG
Pisaurina dubia (Hentz 1847)	50 ± 8	83 ± 15	0.49 ± 0.02	SuF
Pisaurina mira (Walckenaer 1837)	238 ± 12	348 ± 21	0.77 ± 0.03	SuF

Multiple comparisons of mean residual offspring mass and mean residual offspring number were carried out among species within each genus using Tukey-Kramer HSD in order to determine whether and how individual species within a genus differed.

Within species temporal variation. We had samples spanning six or more sampling periods for ten species, and thus we could test for an effect of hatch date on within-species variation in life history traits. Using linear regression adjusting *P*-values for multiple comparisons (the Bonferroni method), we tested for effects of hatch date on female mass, offspring mass, number of offspring, and total clutch mass.

Testing for the effects of interspecific competition. Four ecological communities contained at least four species from the same family. For those communities, we tested the hypothesis that patterns of reproductive allocation would differ among different-sized species within a guild by performing least-squares linear regression, using female mass as the independent variable and reproductive allocation as the dependent variable.

All statistical analyses were carried out using JMP software version 7.0.

RESULTS

Over 3 yr, we collected and analyzed data from 914 individual spiders of 28 species of wolf spider (10 genera) and five species of nursery-web spider (two genera), summarized in Table 1 and in Nicholas et al. (2011).

Phylogeny and reproductive allocation.—The nested analysis of variance showed that most of the variation in reproductive allocation occurred at the family level, rather than generic level. Reproductive allocation was significantly different between families ($F_{1,10}$ = 16.6, P = 0.0005) and explained 60% of the variation in reproductive allocation. Genera nested within families was borderline significant ($F_{10,31}$ = 2.3, P = 0.05) and explained an additional 9% of the variation.

Considering three lycosid genera separately, we found that in each case, species within a genus varied significantly in both residual offspring mass and residual offspring number. Within the genus *Rabidosa,* species category was highly predictive of

Table 2.—Post hoc comparisons of mean residual offspring number (Residuals) within each genus separately. Levels not connected by the same letter are significantly different (Tukey's HSD, $\alpha = 0.05$).

Genus	Species	Levels	Residuals
Hogna	*lenta* B	A	0.375
	annexa	B	0.076
	wallacei	B, C	−0.046
	lenta A	B, C	−0.049
	aspersa	B, C	−0.068
	georgicola	C	−0.069
	watsoni	B, C	−0.361
Rabidosa	*rabida*	A	0.108
	hentzi	A, B	−0.042
	punctulata	B	−0.089
	carrana	A, B	−0.252
Schizocosa	*saltatrix*	A	0.050
	ocreata group	A	0.006
	avida	A, B	−0.003
	duplex	A, B	−0.027
	uetzi	A, B	−0.099
	bilineata	B	−0.328

residual offspring mass ($F_{1,3} = 102.15$, $P < 0.001$) and residual offspring number ($F_{1,3} = 34.57$, $P < 0.0001$). Within the genus *Hogna*, the species category was highly predictive of residual offspring mass ($F_{1,6} = 31.55$, $P < 0.001$) and residual offspring number ($F_{1,6} = 9.31$, $P < 0.001$). Within the genus *Schizocosa*, the species category was highly predictive of residual offspring mass ($F_{1,6} = 10.11$, $P < 0.001$) and less so of residual offspring number ($F_{1,6} = 2.66$, $P = 0.04$). See Table 2 for individual comparisons.

Within-species temporal variation in reproductive allocation.—We examined the relationship between the date of reproduction and female mass, offspring mass, and offspring number among individuals in nine species of wolf spider and one species of nursery-web spider (Table 3). After adjusting for multiple non-independent tests of significance using the Dunn-Šidák method, only one of the 30 regressions was still significant. Further, the mean of the regression slopes was not significantly different from zero for all species combined. The one significant result was for *Hogna lenta sp.* A, where females produced significantly smaller offspring later in the season.

Interspecific competition.—Four ecological communities (see Fig. 1) contained four or more potentially competing species (guilds), that is, species existing in the same habitat type, hatching at a similar time, and observed to feed on the same prey and each other. For each of these four ecological communities (lycosids: SpG, SuF, SuG; pisaurids: SuF), we performed least squares linear regression using reproductive allocation as the independent variable and female mass as the dependent variable to test the hypothesis that reproductive allocation was related to relative body size within a guild (Fig. 1). Among four species of lycosids limited to grassy areas and reproducing in the spring, female mass was positively associated with reproductive allocation, meaning that larger species produced smaller numbers of larger offspring than expected ($r = 0.99$, $df = 2$, $P = 0.01$). For the seven species of lycosids specialized (found only) in forest habitats and reproducing in summer, larger females also produced smaller numbers of larger than expected offspring ($r = 0.83$, $df = 5$, $P = 0.02$). Among fourteen species of lycosids limited to grassy areas and reproducing in the summer, larger species produced smaller numbers of larger offspring than expected ($r = 0.60$, $df = 12$, $P = 0.02$).

Table 3.—Regressions for within season timing of reproduction and the life history traits female mass, mean offspring mass, and offspring number. In each case, time was the independent variable and the life history trait the dependent variable. Sample size (n) was the number of females sampled during the time period. The asterisk denotes the only relationship that was significant after adjusting for multiple tests on non-independent data.

	Species	r^2	n	Sample Period
Female mass	*Pisaurina mira*	0.22	15	22 May–17 June
	Hogna annexa	0.17	20	22 April–11 Sept
	Hogna lenta A	0.39	15	22 May–20 Sept
	Hogna georgicola	0.03	39	8 May–20 Sept
	Pirata A	0.01	8	26 May–27 June
	Schizocosa saltatrix	0.31	7	12 May–16 June
	Pardosa milvina	0.18	14	4 April–8 Aug
	Hogna lenta B	0.47	6	21 Sept–3 Oct
	Varacosa avara	0.21	8	19 April–15 May
	Gladicosa pulchra	0.00	8	3 March–4 April
Offspring mass	*Pisaurina mira*	0.03		
	Hogna annexa	0.01		
	Hogna lenta A	0.51*		
	Hogna georgicola	0.00		
	Pirata A	0.03		
	Schizocosa saltatrix	0.04		
	Pardosa milvina	0.05		
	Hogna lenta B	0.01		
	Varacosa avara	0.00		
	Gladicosa pulchra	0.18		
Offspring number	*Pisaurina mira*	0.46		
	Hogna annexa	0.19		
	Hogna lenta A	0.24		
	Hogna georgicola	0.11		
	Pirata A	0.00		
	Schizocosa saltatrix	0.51		
	Pardosa milvina	0.00		
	Hogna lenta B	0.34		
	Varacosa avara	0.10		
	Gladicosa pulchra	0.04		

However, for the four species of pisaurids also reproducing during the summer and being found only in forested areas, the relationship between adult body size and mean offspring size was negative ($r = -0.84$, $df = 2$, $P = 0.16$). Although the slope is not statistically different from zero, it is strongly negative rather than positive, as in potentially competing groups of lycosids. Further, the slope for pisaurid species is significantly different from the slopes of the three groups of lycosid spiders ($F_{3,23} = 9.60$, $P < 0.05$).

DISCUSSION

We draw three conclusions from our study. First, there is a strong phylogenetic component to the trade-off between offspring size and number among families, within families among genera, and within genera among species. Second, within-season temporal variation in female mass at sexual

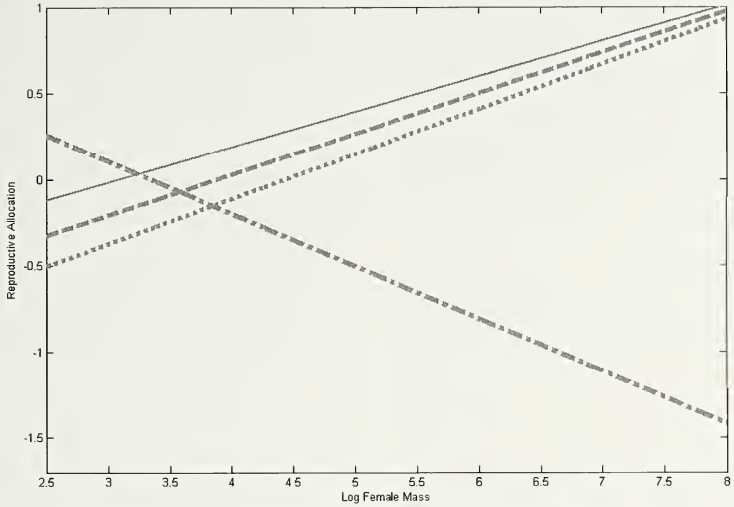

Figure 1.—Linear estimations of the relationship between mean female mass in mg (Log Female Mass) and the principle component axis specifying the trade-off between offspring size and number (Reproductive Allocation). Positive values of the reproductive allocation axis represent species with small numbers of large offspring, and negative numbers represent species with large numbers of small offspring. The three positive slopes represented by the solid, dashed, and dotted lines are all wolf spiders belonging to a particular spider community (SpG, SuF, and SuG respectively), separated in space or time. For wolf spiders, larger species within each guild (SuF) produced relatively few offspring of large size. The negative slope, represented by alternating dashes and dots, represents a guild of nursery web spiders (SuF). Within this community, larger species produced large numbers of relatively small offspring.

maturity, mean offspring mass, and offspring number was observed for only one of the ten species (for offspring mass) for which we had sufficient data. Overall, a clear pattern emerges that female mass, mean offspring mass, and fecundity are constant throughout the breeding season within species. Third, we can draw tentative inferences concerning the effects of competition on reproductive allocation. Female mass was significantly related to patterns of reproductive allocation within potentially competing groups of species (guilds). However, in the Lycosidae larger species within the ecological community produced smaller numbers of larger offspring relative to smaller species. In the Pisauridae, the reverse of this was true, with smaller species producing relatively large numbers of smaller offspring. We elaborate on these conclusions below.

Contribution of phylogeny to patterns of reproductive allocation.—Female reproductive allocation was significantly different between members of the Pisauridae and members of the Lycosidae, showing clear lineage-specific evolution, possibly as the result of different ecological pressures. Family accounted for 60% of the variation in reproductive allocation among genera. The effects of genus nested within family were borderline significant and explained an additional 9% of the variation in reproductive allocation among species. Further, reproductive allocation within the genera *Rabidosa*, *Hogna*, and *Schizocosa* differed significantly among species. The primary result is that members of Pisauridae have significantly larger numbers of smaller offspring than members of Lycosidae. In general, offspring fitness typically increases with offspring size (review in Fox & Czesak 2000 and see Walker et al. 2003 for a specific example with wolf spiders). However, maximizing the fitness of individual offspring does not necessarily maximize the genetic contribution of the parents to the next generation when there is a trade-off between number and size of offspring (Fox & Czesak 2000).

The smaller offspring of the Pisauridae may be favored in part due to the type of maternal care exhibited in this family. Although the wolf spiders examined carry their egg sac on their spinnerets, build a burrow prior to oviposition (G.E. Stratton unpublished), and remain in the burrow until offspring emerge; the pisaurids carry their egg sac in their chelicerae and do not build burrows. Thus, the pisaurids examined in this study are probably more exposed to potential predators, and while carrying egg sacs are prohibited from using their fangs for defense. Numerous researchers have shown that smaller eggs hatch more quickly (e.g., Fox 1997; Azevedo et al. 1996). In this study, pisaurid eggs hatched on average 18 days post-oviposition while lycosid eggs hatched on average 31 days post-oviposition. This earlier hatch time would lessen the period when pisaurid females and young might be most vulnerable to predation. Thus, selection for smaller eggs and faster development times could be an adaptation to this lineage-specific mode of maternal care.

Simpson (1995) found no effect of maternal care on offspring mass or number of offspring among spiders, including members of the Lycosidae and Pisauridae. However,

he placed lycosids and pisaurids in the same category of maternal care, whereas our results suggest that the specific manner of maternal care is correlated with differences in reproductive allocation, suggesting different selective pressures.

We also found significant differences in reproductive allocation within the three genera with sufficient sample size (*Rabidosa*, *Hogna*, and *Schizocosa*) to draw inferences. Our data suggest that life history variation among species is due primarily to interspecific competition and predation within ecological communities (see Importance of interspecific competition below).

Within-species temporal variation in reproductive allocation.—We found little support for temporal changes in reproductive allocation within species during the course of the reproductive season. Statistical power for individual regressions was often low (range: 0.28–0.94), but the fact that the pattern was consistent across all ten species and that the mean slopes were not different from zero strongly suggests that allocation to offspring size and number changes little during the season. Only one species, *Hogna lenta A*, showed a significant seasonal reduction in mean offspring mass (see also Reed & Nicholas 2008). Iida & Fujisaki (2007) showed that females of *Pardosa pseudoannulata* (Bösenberg & Strand 1906) produced smaller numbers of larger offspring late in the reproductive season. Larger offspring have been shown to have higher starvation tolerance and are able to develop more quickly into advanced instars, both of which are traits that have been shown to increase overwintering survival in spiders (Martyniuk & Wise 1985; Iida 2005). *Hogna lenta A*, however, showed the opposite pattern in that larger numbers of smaller offspring were produced late in the reproductive season. It is unclear whether such a reduction is adaptive or perhaps related to a non-significant trend toward smaller females reproducing later in the season.

Importance of interspecific competition.—Our data suggest that interspecific competition, including intraguild predation, might play important roles in the evolution of life history and phenology of species within ecological communities of these spiders. 1) Among three ecological communities of wolf spiders, we found a repeatable pattern of increasing resource provisioning to individual offspring at the expense of numbers of offspring for larger species within guilds. The pattern appears to be the opposite for nursery-web spiders, with larger females producing larger than expected numbers of smaller offspring. However, we have sampled only one such community of nursery-web spiders. 2) Species within the genera *Rabidosa*, *Hogna*, and *Schizocosa* show considerable variation in reproductive allocation and phenology, suggesting niche partitioning within ecological communities and the evolution of divergent phenologies among species within genera to reduce niche overlap. We elaborate on these two points below.

The similar patterns of reproductive allocation among the three communities of wolf spiders (Fig. 1) suggest two alternative explanations: resource partitioning within species among age classes and among species for each age class, or life-history consequences of intraguild predation. The ability for resource partitioning to produce this pattern depends on to what extent spiders switch to larger prey as they grow larger, as compared to just adding larger prey to their prey base at smaller sizes. Zimmerman & Spence (1989) found the former in *Dolomedes triton* (Walckenaer 1837), and Okuyama (2007) found the latter in two species of jumping spider. The same pattern of changes in reproductive allocation with changes in adult size could potentially arise under strong intra-guild predation if the smallest species produce offspring so small that they are below the threshold that triggers predation in larger species, if smaller species produce sufficient numbers of offspring to satiate intra-guild predators, or if sufficiently smaller offspring are too fast for larger species to capture (Rypstra & Samu 2005).

Prior research has indicated that juvenile wolf spiders suffer very high intraguild predation. For five species of wolf spider, other species of spider made up 7.7 ± 0.9% of the diet, with cannibalism accounting for a similar percentage (Hallander 1970; Yeargan 1975; Reed et al. 2007a,b; Reed & Nicholas 2008). Although we have data on only two species, many species within *Rabidosa*, *Hogna*, and *Schizocosa* occupy similar habitats, and all are generalist carnivores, a diet that includes conspecifics as well as congeners (Reed et al. unpublished data). Thus, the potential exists for both competition for resources and competition through intraguild predation to be powerful selective forces. Unfortunately, there are no clear differential predictions for the outcome of resource competition versus intraguild predation.

It is interesting to note that *Hogna lenta* B had an extremely unusual reproductive allocation pattern for a wolf spider. This species is the only grasslands species reproducing in the fall (Table 1), and it produced unusually large numbers of offspring of small size, similar to a pisaurid spider. This provides anecdotal support for the hypothesis that competition and/or the potential for intraguild predation is a major force in the evolution of offspring size, and that the optimum size is quite different under conditions of less intense competition from other cursorial spiders.

The four species of nursery-web spider that form a guild show a very different relationship between female mass and reproductive allocation. In this guild, large species produce unexpectedly large numbers of small offspring. The only detailed study of diet in a nursery-web spider is a study on *Dolomedes triton* (Zimmerman 1989). Other spiders made up 2.9 ± 0.1% of *D. triton's* diet, with almost no cannibalism. The level of intra-guild predation in this one data set is significantly less than for any of the five wolf spider species examined, providing preliminary evidence that guilds of nursery-web spiders generally suffer lower levels of intraguild predation and cannibalism than wolf spiders and, that this could be a contributing factor in the differences in reproductive allocation between the families.

Models of interspecific competition predict competitive exclusion when two or more species reach a certain level of overlap in resource utilization (Hardin 1960; MacArthur & Levins 1967). Hutchinson (1961), however, suggested that competitors with a high degree of overlap in resource utilization could in fact coexist if the competitive advantage shifted seasonally between the competitors. Support for Hutchinson's hypothesis has been shown in several spiders. Balfour et al. (2003) found seasonal shifts in competitive advantage (i.e., predatory dominance) between the wolf spiders *Pardosa milvina* (Hentz 1844) and *Hogna helluo*

(Gertsch 1934). Spiller (1984) found a similar shift between two species of orb-weaving spider, *Metepeira grinnelli* (Coolidge 1910) and *Cyclosa turbinate* (Walckenaer 1842). Our results suggest that competitive and predatory interactions may select for asynchronous phenologies as well as influence the pattern of reproductive allocation among the species examined.

Rabidosa rabida, *R. hentzi*, and *R. punctulata* are all found in open grasslands, and all three exploit resources in a similar manner, climbing to the top of grass stems to wait for arthropod prey. There is a high degree of diet overlap between *R. punctulata* and *R. rabida* (niche overlap on the diet axis between these two species is 0.93; Reed et al. unpublished). The heavy overlap in resource utilization among these species creates the potential for intense interspecific competition. Detailed field observations over a three-year period suggest that asynchronous phenology and differences in reproductive allocation may provide an important mechanism allowing coexistence among these members of *Rabidosa* (Reed and Nicholas 2008). However, whether the differences in phenology and reproductive allocation observed in *Rabidosa* evolved due to competition or are a prior adaptation that allows coexistence among these species is unknown. Future work involving manipulation of species composition in experimental plots is needed (Connell 1980).

We have shown that reproductive allocation with respect to offspring size and number is significantly different between the closely related families Lycosidae and Pisauridae. Further, we show that despite strong phylogenetic conservatism among genera within a family, species within genera are varied in their allocation of reproductive resources and apparently respond to differential selection pressures for the offspring size and number continuum. In particular, intraguild competition and predation may be important factors impacting cursorial spider life history evolution and community structure. However, conclusions concerning the importance of completion are tentative and will require further research.

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An old lineage of Cyphophthalmi (Opiliones) discovered on Mindanao highlights the need for biogeographical research in the Philippines

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Abstract. The arachnid order Opiliones, and the suborder Cyphophthalmi in particular, have recently been used to test biogeographical patterns in Southeast Asia due to their ancient age and extremely low vagility. Here we report the first Cyphophthalmi—two juveniles—known from Mindanao in the southern Philippine Archipelago, and we place them in a molecular phylogeny to test biogeographical hypotheses for their colonization of that island. Five molecular markers were sequenced from one specimen, three from the other, and these sequences were added to a previously completed phylogenetic analysis. The specimens were recovered as members of a clade found almost exclusively on Borneo. Their deep placement within this clade suggests a very old origin and colonization that perhaps involved the mysterious landmass now underlying Mindanao's Zamboanga Peninsula. This species prompts new questions about the abilities of Southeast Asian Cyphophthalmi (Stylocellidae) to disperse and colonize, and it emphasizes how much remains to be understood about the geological history of the Philippines.

Keywords: Southeast Asia, Borneo, Zamboanga, harvestmen, stylocellidae, biogeography

Cyphophthalmi is a suborder of Opiliones, and most of its members are exceptionally poor dispersers. Species have highly constrained ranges (Giribet 2000), or, if widespread, demonstrate little gene flow between populations (e.g., Boyer et al. 2007a for *Aoraki denticulata* [Forster 1948]; R. Clouse unpublished data for *Metasiro americanus* [Davis 1933]). The Southeast Asian Cyphophthalmi, all in the family Stylocellidae, have been shown to have a few cases of trans-oceanic dispersal (Clouse & Giribet 2007), but their phylogeny matches hypothesized geologic events close enough to suggest that their present distribution is mostly due to vicariance (Clouse & Giribet 2010), as is characteristic for the suborder as a whole (Boyer et al. 2007b).

The biogeography of Southeast Asia is commonly noted for the region's distinct biotic boundaries, and before the acceptance of continental drift, these breaks were the first clues that landmass configurations had changed dramatically over time (Wallace 1859; Simpson 1977). Today most of these biotic breaks are seen as collective limits for organisms of similar origins, dispersal capabilities, and ecological requirements (Mayr 1944), but some have also been shown to have dubious meaning altogether. In the latter category is Huxley's line, which separates Borneo and Palawan (Fig. 1) from the remainder of the Philippines; it was based on the range limits for certain avian species, particularly megapodes and pheasants (Huxley 1868).

Huxley's line does mostly separate continental landmasses (Borneo and Palawan) from those of oceanic and volcanic origins, although this appears to be incomplete, coincidental, and rather meaningless vis-à-vis biogeographic questions. Palawan is hypothesized to be continental crust moving south from the Chinese coastline with the opening of the South China Sea, but along with it likely came Mindoro and perhaps even parts of Zamboanga (Fig. 2) (Yumul et al. 2004), which Huxley grouped with the volcanic Philippine islands. In addition, Palawan's positioning close to (and perhaps connected to) Borneo is a relatively recent phenomenon, happening only in the past 10 million years, in contrast to various volcanic formations that formed earlier off the coast of Borneo and are now part of the Philippine Archipelago (Hall 2002; Yumul et al. 2009). Cases of lineages of organisms that cross Huxley's line have made obsolete the notion that Philippine biogeography is best understood by a single biotic break between it and Borneo, and Palawan in particular has been shown to play different roles for different lineages (Essylstyn et al. 2010; Oliveros & Moyle 2010; Siler et al. 2010). Crossings of Huxley's line are especially interesting when looking at poor dispersers, like freshwater amphibians. For example, Southeast Asian stream frogs (*Rana signata* complex) have apparently invaded the Philippines from Borneo via Palawan and Mindoro, as well as possibly through the Sulu Archipelago and Mindanao (Brown & Guttman 2002), and oriental stream toads (*Ansonia*) appear to have crossed Huxley's line from Borneo to Mindanao (Matsui et al. 2010).

Cyphophthalmi, perhaps the least vagile animals in the region, have previously appeared to occur only west of Huxley's line, having been described from Palawan Island and Borneo but not from the remainder of the Philippines (Shear 1993; Clouse et al. 2009), but here we report the first Cyphophthalmi (Figs. 3–8) from the island of Mindanao in the southern Philippines, yet another exception to this supposed faunal break. We have previously reported a firsthand account of perhaps seeing Cyphophthalmi from Luzon by P. Schwendinger (Clouse & Giribet 2007), but further information or a specimen has not been available. Our objective upon finding the Mindanao specimens was to narrow the possible scenarios for their origin by placing them

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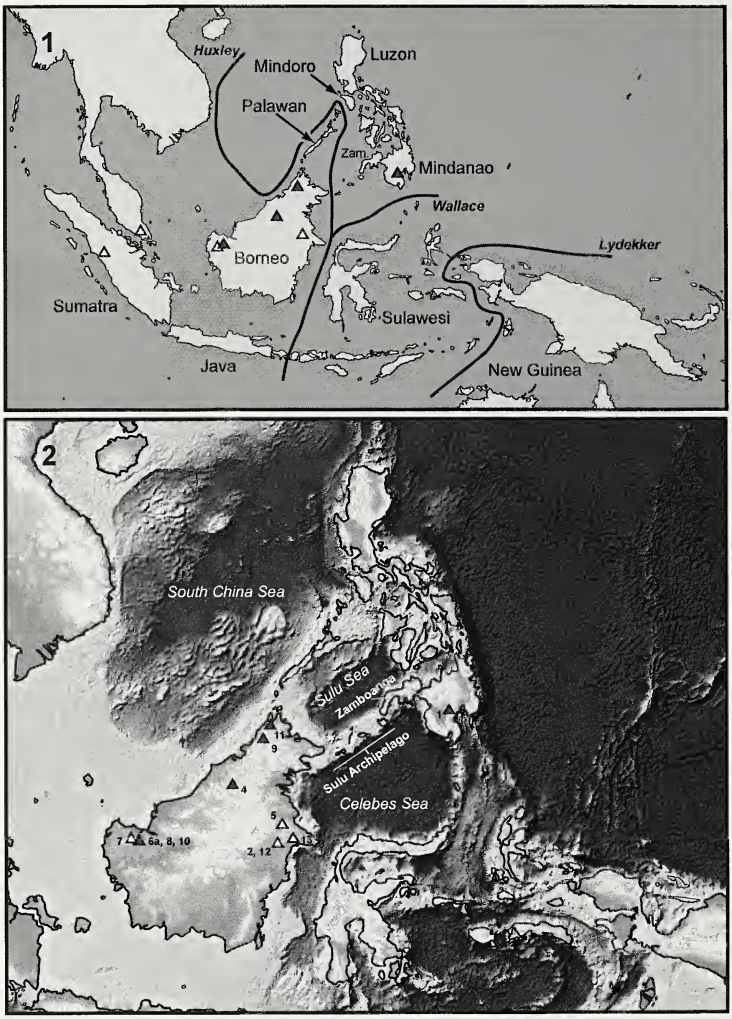
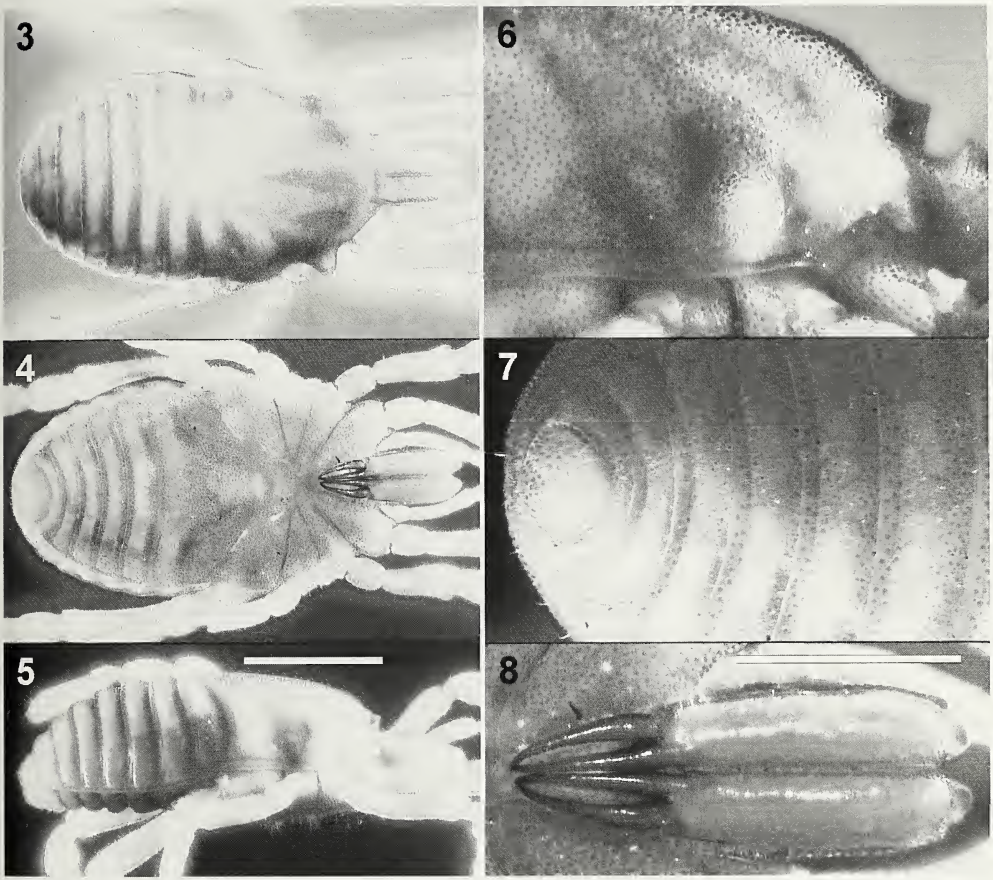


Figures 1, 2.—Southeast Asia, showing *Miopsalis* localities, with species from clades I and II designated by open triangles and from clade III by filled (black) triangles. 1. Biotic breaks demarcated by Huxley, Wallace and Lydekker; "Zam." = Zamboanga Peninsula on western Mindanao; 2. The topography and bathymetry of the northeastern Malay Archipelago.

in a recently completed, dated phylogeny of the Southeast Asian Cyphophthalmi (all in Stylocellidae) (Clouse & Giribet 2010). From this phylogeny, we have inferred that stylocellids arrived in Southeast Asia on the Sibumasu terrane, which rifted from Gondwana in the late Paleozoic; the genus *Fangensis* is an old lineage in the family and still found exclusively on the Sibumasu. From there, the genus *Meghalaya* extended north as far as northeast India and China's Yunnan Province, and then, after the appearance of Borneo in the late Mesozoic, the genus *Miopsalis* expanded into that landmass while it was still connected to the Thai-Malay Peninsula. A fourth and final clade, *Leptopsalis*, diversified over the whole southern end of the once-unified Sundaland Peninsula (and into eastern Thailand; see Clouse & Giribet 2010:fig. 1) before it broke apart into today's Indo-Malay Archipelago, carrying stylocellid lineages presently found on



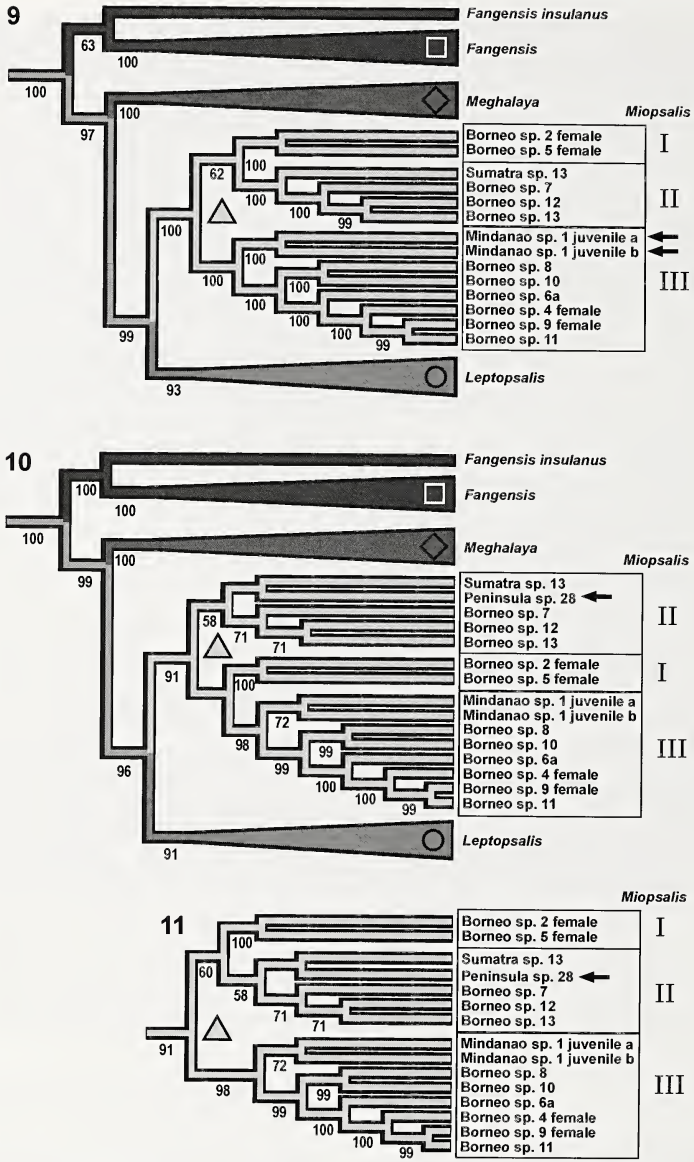
Figures 3–8.—A juvenile cyphophthalmid collected from Mindanao Island, Philippines. 3. Dorsal; 4. Ventral; 5. Lateral; 6. Lateral anterior; 7. Ventral posterior; 8. Chelicers. Scale bars equal 1 mm (Figs. 3–5); 0.50 mm (Figs. 6–8).

Java, Sumatra, and Sulawesi. (The remaining genus in the family, *Stylocellus*, which currently contains the bulk of the named species in the family, has not had its type specimen placed reliably in the four main lineages [Clouse et al. 2009].) Before sequencing the Mindanao species we were unsure if it would fall in *Miopsalis*, found almost exclusively on Borneo, or in *Leptopsalis*, which is found throughout the Indo-Malay Archipelago, including Northern Sulawesi directly to the south. Northern Sulawesi *Leptopsalis* are also related to species on New Guinea (Clouse & Giribet 2007), indicating a possible proclivity for dispersal in that group.

METHODS

On December 15–16, 2009, leaf litter that was later found to have two juvenile cyphophthalmids was collected from the following location: Barangay (village) Kimlawis, Municipality of Kiblawan, Davao del Sur Province, in the central region of Mindanao (estimated coordinates: 06.47836°–06.48528°N, 125.08317°–125.08689°E). The litter was collected from two remnant patches of degraded, logged-over, lowland forest at about 500 m above sea level.

The specimens are presently stored at −80° C in 95% EtOH in the Department of Invertebrate Zoology at the Museum of Comparative Zoology (Harvard University) under collection number MCZ DNA104981. We attempted to sequence fragments of 16S rRNA (~470 bp), 18S rRNA (~1760 bp), 28S rRNA (~2100 bp), cytochrome *c* oxidase subunit I ("COI," ~814 bp), histone H3 (327 bp), and histone H4 (160 bp). Only 16S rRNA did not amplify for either specimen, and the smaller specimen did not produce sequence data for COI or histone H3. Completed sequences are deposited in GenBank under accession numbers HQ593865–HQ593872.



Figures 9–11.—Phylogenetic hypotheses for the placement of the juvenile Mindanao cyphophthalmids (sp. 1a and 1b, arrows). Collection symbols (square, diamond, triangle, circle) and species monikers match Clouse and Giribet (2010). The clades *Fangensis*, *Meghalaya*, and *Leptopsalis* have been collapsed, with the exception of *F. insulanus*, which is often recovered as sister to the non-*Fangensis* stylocellids. *Fangensis* is found in the northern and central parts of the Thai-Malay Peninsula, *Meghalaya* in the Thai-Malay Peninsula and Eastern Himlayas, and *Leptopsalis* in the Indo-Malay Archipelago. Support values under each node are jackknife values using original data partitions. 9. The phylogeny does not include the terminal "Peninsula sp. 28" and is 24,304 weighted steps long. 10. The shortest tree (24,341 weighted steps) including Peninsula sp. 28 (arrow), which caused clade I to become sister to III. 11. With "Peninsula sp. 28", there was higher jackknife support for the original position of clade I as sister to II.

We used a recently completed comprehensive study of Southeast Asian Cyphophthalmi (Clouse & Giribet 2010) to place the Mindanao species in a large phylogeny efficiently. This phylogeny was comprised of six non-cyphophthalmid Opiliones, 21 non-stylocellid Cyphophthalmi, and 95 Stylocellidae, representing the Eastern Himalayas, the Thai-Malay Peninsula, Sumatra, Borneo, Sulawesi, Java, and New Guinea. First, we added the Mindanao terminals as basal branches to one of the shortest trees found earlier under each of nine different transformation cost schemes. We then applied traditional branch swapping as well as a genetic algorithm to those nine trees using POY version 4.1.2 (Varón et al. 2009) on 20 parallel nodes under the previous study's optimal cost scheme ("121," where indels and transversions cost two and transitions cost one). This addition of terminals to previously found trees is akin to Mecham et al.'s (2006) "jumpstarting" and Giribet's (2007) "pre-processed searehes." After finding the shortest trees containing the Mindanao terminals, we added the critical terminal "Peninsula sp. 28" from Kota Tinggi, Johor, Malaysia, and did another round of searching. "Peninsula sp. 28" is known from a single specimen, has only the 18S rRNA fragment and less than half of the 28S rRNA fragment sequenced, but morphologically it resembles other species in the genus *Miopsalis* and was recovered there in previous phylogenetic searches.

Nodal support was evaluated with 100 jackknife pseudoreplicates, each starting from trees found earlier under cost schemes 111 (all transformations equal) and 441 (indels cost 16, transversions cost four, and transitions cost one), and with the Mindanao and "Peninsula sp. 28" terminals added as basal branches. Dynamic homology was used during the jackknife searches, with the data fragmented into the same partitions used during the original tree searches. The jackknife removal percentage, which in the dynamic homology context refers to the percent of data partitions randomly removed to generate each pseudoreplicate, was set at 0.36 (Farris et al. 1996). Clades are here referred to by their tentative genus names pending an ongoing revision of the family (see Clouse 2010).

Dates for the origins of the Mindanao species were approximated from the dates estimated earlier for Stylocellidae (Clouse & Giribet 2010). That analysis was done by setting the root to 425 Ma and making nodal date estimates in the program r8s 1.71 (Sanderson 2003). The date for the root was based on an early Devonian fossil opilionid (Dunlop et al. 2004) and the hypothesis that Opiliones are sister to Scorpiones, for which mid-Silurian stem-group fossils are known (Giribet et al. 2002; Dunlop et al. 2007).

RESULTS

Despite juveniles lacking important taxonomic characters, morphology initially suggested that the Mindanao specimens are stylocellids: presence of a solea (concentration of setae) on tarsus of leg I, ornamented tarsi in all legs, coxa of leg II fused to coxae III–IV, C-shaped tracheal spiracles, and sculpturing on the second cheliceral article (Giribet 2002). This was supported by our molecular analysis. Within Stylocellidae, the two Mindanao specimens (likely the same species) placed inside the genus *Miopsalis* as sister to ones found exclusively on Borneo (Fig. 9, clade III). When "Peninsula sp. 28" was added, that species placed inside clade II as sister to the Sumatran species (Fig. 10). "Peninsula sp. 28"'s inclusion also caused clade I to become sister to clade III, but there was actually 60% resampling support for the original position of clade I as sister to clade II (Fig. 11). This general arrangement of clades within *Miopsalis*, as well as the close placement of Sumatran and Peninsular species inside clade II, match results from our earlier analyses (Clouse & Giribet 2010). Previously we estimated that clades (I + II) and III split 168 Ma, that clade III (without the Mindanao species) started diversifying around 100 Ma, and clades I and II split at 116 Ma. Our best-supported phylogenies (Figs. 9, 11) suggest that the Mindanao lineage originated between 100 and 168 Ma, from the Middle Jurassic to the Early Cretaceous. The shortest tree with "Peninsula sp. 28" supports the earlier end of this estimate, between 100 and 116 Ma.

The phylogenetic results closely matched our previous hypotheses constructed before the Mindanao species was discovered (Clouse & Giribet 2010), with the one exception of *Fangensis* being recovered as monophyletic (Figs. 9, 10) in the shortest tree found under the optimal parameter set. However, this result was not surprising, often being found under different parameter sets, and well-supported, stable clades among the other 122 terminals were recovered again here.

DISCUSSION

The Mindanao species could have arrived by transoceanic dispersal, especially since the old age of this lineage improves the chances of encountering rare dispersal events. Stylocellidae may also have both intrinsic and external advantages in surviving open seas and colonizing coastal areas (i.e., participating in taxon cycles according to Wilson 1961). The large sizes of many species (especially on Borneo) and highly sclerotized cuticle may help prevent desiccation, and their well-developed eyes may help them find their way out of suboptimal conditions. Furthermore, the presence of many islands throughout Southeast Asia may minimize their time spent at sea relative to other regions.

Nonetheless, any route that maximizes contact with humid leaf litter under a closed canopy (Cyphophthalmi's exclusive habitat, with a few exceptions of subterranean environments) would be the most likely one used by the Mindanao species from Borneo. Two commonly proposed routes to Mindanao, whether by island hopping or land bridges, are 1) via Palawan, Mindoro, and the volcanic islands of the Philippine Archipelago, and 2) via the Sulu Archipelago. However, the Mindanao species represents a very old lineage, and the conditions for land bridges over these two routes (Palawan's arrival and eustatic extremes) have been recent (Yumal et al. 2009). Old lineages can still have recent dispersal events, but a second problem is that the Mindanao species are most closely related to species in western Borneo (8, 10, and 6a), not, as one would expect, to the ones in closest proximity (Figs. 1, 2, 9–11).

A third possible route to Mindanao, especially for old lineages, may come from the Zamboanga Peninsula, which appears to have been in closer proximity to Borneo for a longer period of time than the remainder of Mindanao. Hall (2002) reconstructed Zamboanga as having arisen near northeastern Borneo 50 Ma and only moving away to join the remainder of Mindanao in the past 5 Ma. Explicit

reconstructions of land exposure for arc, ophiolitic, and accreted material by Hall (1998, 2001) also show Zamboanga and Mindanao as having small areas above sea level around volcanoes as far back as at least 30 Ma, although hypotheses of exposure for any landmass in Southeast Asia, especially in the Cenozoic, are accompanied by considerable uncertainty (Voris 2000; Lambeck & Chappell 2001). In 2002, Hall noted evidence for continental material in Zamboanga but also the newness and variable quality of data on Philippine geology, adding yet more intrigue and uncertainty to its history. Geologic data on Zamboanga has since improved, and some see a strengthening case for it having once been part of Palawan (Yumul et al. 2004). What is clear is that the history of Mindanao is far from settled, and the door is open to ancient colonizations or range expansions into Zamboanga before the remainder of Mindanao formed.

Matsui et al. (2010) dated the split between Bornean and Mindanao stream toads at 39 Ma and between two Mindanao species at 20.2 Ma, leading them to argue against their methods in order to avoid the unlikely scenario of two invasions of Minadano over Pleistocene land bridges, which formed more than 18 million years later. Blackburn et al. (2010) also found very old dates for the origin of flat-headed frogs on Palawan and Borneo, prompting them to invoke a "Palawan Ark" rafting scenario. For the Mindanao stylocellids, our phylogenetic and dating estimates would need to be highly erroneous to push their origin from the Mesozoic to the Pleistocene, and so we have explored other options to explain their odd occurrence. Zamboanga appears to offer new possibilities for explaining past crossings of Huxley's line, although much work remains to clarify its role in Southeast Asian biogeography. If species dispersed directly from Borneo to proto-Zamboanga, this should be quite discernible in species distributions and phylogenetic analyses as more Cyphophthalmi are discovered in the region.

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The natural diet of a polyphagous predator, *Latrodectus hesperus* (Araneae: Theridiidae), over one year

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Abstract. The natural diets of many terrestrial predators such as spiders have yet to be investigated. In this study, I analyzed the diet of a web-building spider, *Latrodectus hesperus* Chamberlin & Ivie (1935), over one year in a natural habitat of coastal British Columbia, Canada. This is the first study to document the natural diet of *L. hesperus* over several months. I identified and measured 1599 prey items collected from *L. hesperus* webs and web sites between January and December. Spiders fed on ground-active prey from eight different orders of arthropods. Coleoptera and Hymenoptera were the predominant prey of *L. hesperus* in this habitat, combinely accounting for > 85% of the total prey catches and biomass. The other prey orders included, in order of abundance, Isopoda, Araneae, Dermaptera, Orthoptera, Lepidoptera and Diptera. Spiders captured prey mostly between May and October, when females oviposit, juveniles grow, and prey are most active. These results show that *L. hesperus* is a polyphagous predator that feeds primarily on prey from two orders of insects.

Keywords: Feeding regime, foraging, predator-prey interactions, prey, spiders

An animal's diet breadth typically falls along a generalist-specialist continuum. One extreme is represented by generalist foragers that feed on a variety of organisms from different taxonomic groups; the opposite end consists of specialists that feed exclusively on a single type of organism or taxon, even when others are available to them. Most animals fall somewhere in between the two depending on the environment they live in and their foraging strategies (Futuyma & Moreno 1988).

Much research on animal diets has focused on terrestrial arthropods, and has documented the evolution of diverse patterns of resource use involving herbivory, predation and parasitism (Nentwig 1987; Jaenike 1990; Bernays & Minkenberg 1997). Spiders are important terrestrial predators that sit at the top of many invertebrate food webs and show varied feeding habits. They are for the most part polyphagous and prey upon a variety of invertebrate taxa across a broad range of habitats (Nentwig 1987; Riechert & Harp 1987). Yet, a few species specialize on prey, such as ant-eating zodariid spiders, araneophagic mimetid spiders, and moth-eating araneid spiders (Jackson & Whitehouse 1986; Stowe 1986; Pekár 2004).

A balanced diet composed of different prey types may be adaptive for spiders. Indeed, polyphagy provides access to a variety of nutrients not available from a single prey source, which may maximize growth rates and juvenile survival (Uetz et al. 1992; Toft & Wise 1999). However, a mixed diet may be constrained by the habitat-dependent availability of certain prey types. Under such constraints, spiders can maximize diet quality by selectively feeding on particular subsets of prey in the environment that may be abundant or highly nutritious (Riechert & Harp 1987; Futuyma & Moreno 1988).

Two empirical methods have commonly been used to study the feeding habits of spiders; both have provided ample evidence of the polyphagous nature of many spider species. The first one involves feeding experiments with different assortments of prey. The results of such experiments have shown that some spiders feed indiscriminately on different prey types, while others show preferences for certain prey types based on particular morphological or behavioural attributes of the prey (e.g., Nentwig 1986; Toft & Wise 1999; Pekár 2004). The second method is used to characterize the actual range of prey consumed by a particular species in its natural habitat based on field surveys and observations (e.g., Robinson & Robinson 1970; Hódar & Sánchez-Piñero 2002; Guseinov 2006). Collectively, these field studies have shown that a spider's diet breadth may depend on its foraging strategy and the type of habitat it lives in. Given the great diversity of spiders, more studies in natural settings are needed to determine what a species *does* eat in relation to what it *can* eat.

The aim of this study is to characterize the diet of a locally abundant web-building spider, *Latrodectus hesperus* (Chamberlin & Ivie 1935) (Araneae: Theridiidae), over one year in a natural habitat of southwestern Canada. I collected and identified all prey items of *L. hesperus* spiders each month and analyzed their diet based on prey composition and numbers, prey size, prey biomass and prey-capture rate.

METHODS

Study area.—This study was conducted in a coastal sand dune habitat of southern Vancouver Island, British Columbia, Canada (48°34′N, 123°22′W, elev. 2–3 m), in an area located above the high-tide line and ~ 90 m from the shore. The study site was a ca. 600-m^2 area of open sandy habitat with interspersed clusters of driftwood logs, bordered by densely-spaced trees and shrubs (see Salomon et al. 2010 for details). The weather at this site is cool and wet from October–March and both warmer and drier between April–September.

Study species.—*Latrodectus hesperus* is a web-building spider that is native to western North America and found from Mexico to southwestern Canada (Kaston 1970). At the study site, *L. hesperus* is the dominant web-building spider. Furthermore, individuals are facultatively group living, i.e., they occur either solitarily or in small groups depending on habitat conditions and time of year (Salomon et al. 2010).

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Spiders live exclusively under driftwood logs found throughout the open sandy habitat and build three-dimensional cobwebs on the underside of the logs. Their webs are often quite extensive and have a central tangle region from which sticky 'gumfooted' silk lines designed to capture prey extend vertically to the ground.

General setup and prey-sampling method.—Thirty rectangular wooden sheds were placed in and around a large cluster of driftwood logs at the study site in early January 2003 as part of a 3-yr study of group living in *L. hesperus* (see Salomon et al. 2010). These sheds provided new habitat in which wandering *L. hesperus* spiders could establish themselves. The sheds were built with two 150 × 14 cm cedar boards that were orthogonally nailed together, and their dimensions corresponded to those of an average-sized driftwood log occupied by *L. hesperus*. *Latrodectus hesperus* spiders readily settled under the sheds and their populations persisted over several years (Salomon et al. 2010). This semi-natural setup was ideal for studying the diet of *L. hesperus*, as it provided uniform habitat space in which it was possible to reliably sample prey.

The current study was conducted from January–December 2005. By the time it was initiated, *L. hesperus* spiders were well established under the sheds and occupied 80–100% of the sheds year-round.

I counted the total number of *L. hesperus* spiders under each shed on a monthly basis in 2005 and collected their prey and identified them. In late December 2004, I cleared all prey remains from *L. hesperus* webs and the sandy substrate under the sheds. Starting in late January 2005 and continuing on a monthly basis until December, I collected all prey items that had been captured by spiders in the preceding month. This was done by carefully picking prey off the webs (unless spiders were still feeding on them) and collecting discarded prey from the substrate under the sheds. This protocol represents a very effective method of collecting prey of *L. hesperus*, yielding most, if not all, prey items. Two other web-building species co-occurred with *L. hesperus* under the sheds at low densities: *Tegenaria agrestis* (Walckenaer 1802) and *T. duellica* (Simon 1875) (Araneae: Agelenidae). Unlike *L. hesperus*, *Tegenaria* spiders usually macerate and compact their prey during consumption, rendering most remains unrecognizable as prey (extensive laboratory feedings with *T. agrestis and T. duellica* have shown that individuals practically always macerate and compact prey from various taxa; S. Vibert, unpublished data). I only collected prey items that were still whole or broken into recognizable pieces. It is thus very likely that most, if not all, of the collected prey were those of *L. hesperus* spiders because the integrity of their prey is preserved after consumption. I identified all prey items to order level under a stereo microscope and used various taxonomic keys as references.

Prey-capture metrics.—I quantified the number and proportion of prey from different arthropod orders that spiders captured each month, and determined prey composition as the diversity of prey orders captured. The degree of variation in prey composition was quantified using Levins' standardized index of diet breadth, $B_A = \left((1/\sum p_i^2) - 1\right)/(n-1)$, where p_i is the proportion of prey items from prey type i, and n is the total number of prey types (Hurlbert 1978; Krebs 1999). This index ranges from 0 to 1, with values close to 0 indicating that a predator consumes few prey types in high proportion, and values close to 1 indicating that all prey are consumed in equal proportion. Note that this index does not account for differences in prey type availability in the habitat, which was not measured and thus cannot be controlled for in the analyses. I calculated monthly B_A values as well as an overall value for the whole study period. I also computed the inverse Simpson's index of diversity, $1/D = 1/\sum p_i^2$, which ranges from 1 to the total number of prey types, with higher values representing a greater diet breadth (Krebs 1999).

Prey size and biomass.—For all except Araneae (spider) prey, I measured the total body length of each prey item with digital callipers (to the nearest 0.01 mm) and used these data to calculate dry mass based on taxonomic order-specific regression equations available from the literature (see Appendix 1). Araneae prey were not always intact (e.g. some had deformed abdomens), so I measured the combined length of the tibia and patella of their first pair of legs (a reliable index of size in spiders; Jakob et al. 1996) instead of their total body length. The dry mass of Araneae prey was then calculated using regression equations developed for each of the three types of Araneae prey collected under the sheds: *Tegenaria* spp. (*T. agrestis* and *T. duellica*), *Latrodectus hesperus*, and Lycosidae. Only two Araneae specimens did not belong to these categories (1 salticid and 1 antrodiaetid spider; see Results); for these I used the regression equation developed for Lycosidae, which was judged to be sufficiently accurate for the purpose of this study.

To calculate dry mass from body size in *Tegenaria* spp. and *L. hesperus* prey, I developed two regression equations: a first one relating body size to wet mass and a second one relating wet mass to dry mass (Appendix 1). For the first equation, I measured the tibia-patella length of leg pair 1 (in mm; precision: 0.01 mm) and wet mass (precision: 0.1 mg) of 86 *L. hesperus* and 28 *Tegenaria* spp. (15 *T. agrestis* and 13 *T. duellica*) field-collected adult females, regressed both variables, and determined the fit of the regression using a General Linear Model (GLM). For the second equation, I weighed 32 *L. hesperus* and 16 *Tegenaria* spp. (8 *T. agrestis* and 8 *T. duellica*) field-collected adult females, killed them by freezing, dried them in an oven at 60 °C for 96 h, and re-weighed them once fully dry. From these wet mass data I calculated dry mass using a regression equation. To derive dry mass from body size in Lycosidae prey, I developed a single regression equation based on data from four species of lycosid spiders (n = 32; 8 specimens each of *Alopecosa kochi* (Keyserling 1877), *Arctosa perita* (Latreille 1799), *Pardosa* spp., and *Trochosa terricola* (Thorell 1856)) collected in pitfall traps around the study site from March–June 2003 as part of a separate study (M. Salomon & R.G. Bennett, unpublished data). I measured the tibia-patellar length of the first pair of legs of each spider, dried them using the protocol described above and weighed them when fully dry.

I used a General Linear Mixed Model (GLMM) to test for variation over time in average prey length per shed (log-transformed) based on data from all except Araneae prey, with month as a within-subject factor and shed identity as a subject factor.

RESULTS

Diet breadth.—The overall diet breadth of *L. hesperus* at the study site was 0.18 (standardized Levins' index, B_A), indicating

Table 1.—Prey of *Latrodectus hesperus* spiders in coastal British Columbia, Canada, between January–December 2005.

Prey taxon	Total number	% Total number	Total biomass (dry g)	% Total biomass	Body length (mm) (mean ± SD (range))
Insects					
Coleoptera	974	60.91	2953.94	87.81	8.35 ± 2.28 (4.66–24.19)
Hymenoptera	422	26.39	335.35	9.97	10.02 ± 4.39 (4.97–21.70)
Dermaptera	32	2.00	2.32	0.07	10.36 ± 1.60 (6.14–13.20)
Orthoptera	25	1.56	21.73	0.65	17.66 ± 4.29 (10.34–25.71)
Lepidoptera	15	0.94	11.50	0.34	17.18 ± 3.61 (13.64–28.26)
Diptera	5	0.31	0.83	0.03	10.76 ± 0.91 (9.42–11.74)
Malacostraca					
Isopoda	69	4.32	18.95	0.56	9.06 ± 1.30 (6.01–11.44)
Arachnids					
Araneae	57	3.57	19.54	0.58	–
TOTAL	1599	100.00	3364.16	100.00	–

that spiders preyed upon a few arthropod orders in high proportion and many orders in small amounts. Monthly B_A values ranged from 0.04 (in March) to 0.23 (in July) with a median of 0.16 from January–December. Overall diet breadth expressed as the inverse Simpson's index ($1/D$) was 2.25, and ranged from 1.25 (in March) to 2.62 (in July) with a median of 2.10. This means that *L. hesperus* fed predominantly on 2 prey orders.

Prey composition, size and biomass.—Between January and December, I collected and identified 1599 prey of *L. hesperus*. The diet of *L. hesperus* was composed of prey from 8 different orders of arthropods present in variable quantities (Table 1; Fig. 1a,b). Spiders fed primarily on beetles (order Coleoptera) that varied widely in body length, and these represented > 60% of all prey catches and > 80% of the total prey biomass (Table 1). The main types of Coleoptera prey were, in order of abundance: tenebrionid, curculionid and carabid beetles.

The second most abundant prey order was Hymenoptera, which included ants (Formicidae; 52.4% of Hymenoptera prey), sand wasps (*Bembix* sp. (Sphecidae); 26.1%), paper wasps (*Polistes* sp. (Vespidae); 10.4%), bumble bees (*Bombus* sp. (Apidae); 5.9%), ichneumonid wasps (Ichneumonidae; 4.0%), honey bees (*Apis* sp. (Apidae); 0.7%), and other sphecid wasps (Sphecidae; 0.5%). The smallest hymenopteran prey were ants and the largest were paper wasps (Table 1); the overall prey-size distribution of hymeopterans was bimodal with many large (wasps and bees; median length: 14.1 mm) and many small prey (mostly ants; median length: 6.0 mm).

The remaining orders of arthropod prey each represented < 5% of the total prey catch and <1% of the total prey biomass (Table 1). These included, in order of abundance as prey, Isopoda, Araneae, Dermaptera, Orthoptera, Lepidoptera and Diptera. Spiders that were preyed upon included wolf spiders (Lycosidae, 77.2% of Araneae prey; primarily *Alopecosa kochi*, *Arctosa perita*, *Pardosa* spp. and *Trochosa terricola*), *T. agrestis* and *T. duellica* adults and juveniles (12.3%), *L. hesperus* adults, subadults and juveniles (7.0%), 1 male *Habronattus americanus* (Keyserling 1885) (Salticidae) and 1 female *Antrodiaetus pacificus* (Simon 1884) (Antrodiaetidae). Lycosid prey were 0.4–0.9 × the average size of adult female *L. hesperus* (mean ± SD tibia-patellar length of field-collected females: 6.46 ± 0.33 mm, $n = 86$), whereas *Tegenaria* prey were 0.8–1.7 × the average size of adult female *L. hesperus*. Salticid and antrodiaetid prey were 0.3 and 0.9 × the average size of adult female *L. hesperus*, respectively.

Overall, the distribution of prey lengths (i.e. all except Araneae) varied over time in accordance with the availability of different types of prey (GLMM: $F_{11,213.9} = 2.93$, $P = 0.001$; Fig. 1c). There was no clear seasonal pattern in prey-length distributions. Median prey length was highest in October (9.7 mm) and lowest in November (6.9 mm). The large majority of prey (90%) were 6–14 mm in length, i.e. 0.5–1.3 × the average body length of adult female *L. hesperus* (females are generally 10.5–13 mm in length; Kaston 1970).

Timing of prey capture.—*Latrodectus hesperus* spiders captured prey year-round (Fig 1a), but most prey (78.9%) were captured from May–October when females produce egg sacs and emerging juveniles grow and mature (Fig. 1d; see also Salomon et al. 2010). Most prey orders showed temporal variation in the catch (Fig. 2). Coleoptera varied in abundance over time in the prey catch but were the dominant prey each month. Hymenoptera were common prey only from May–September, which corresponds to their season of peak activity at the study site (pers. obs.; Figs. 1a, 2). Sand wasps and bumble bees were captured during an even shorter time window, i.e. June–August. Other prey orders such as Isopoda and Orthoptera showed a peak in abundance between July–October (Fig. 2). *Latrodectus hesperus* fed upon con- and heterospecific spiders at a relatively constant rate, with a peak of predation on lycosids in April (Fig. 2).

DISCUSSION

The results of this study show that the diet of the web-building spider *L. hesperus* in coastal British Columbia, Canada, is characteristic of a polyphagous predator. *Latrodectus hesperus* spiders fed on eight different orders of ground-active arthropods, captured mostly from May to October, which is the period of oviposition and peak juvenile growth, when population densities are highest (Salomon et al. 2010). However, spiders were mostly insectivorous with two insect orders (Coleoptera and Hymenoptera) as their primarily sources of prey. Of the two, Coleoptera made up the large majority of prey catches and especially prey biomass.

The diet breadth of *L. hesperus* is consistent with that of other web-building spiders (reviewed in Nentwig 1987 and Nyffeler 1999). Most web-building spiders are broadly

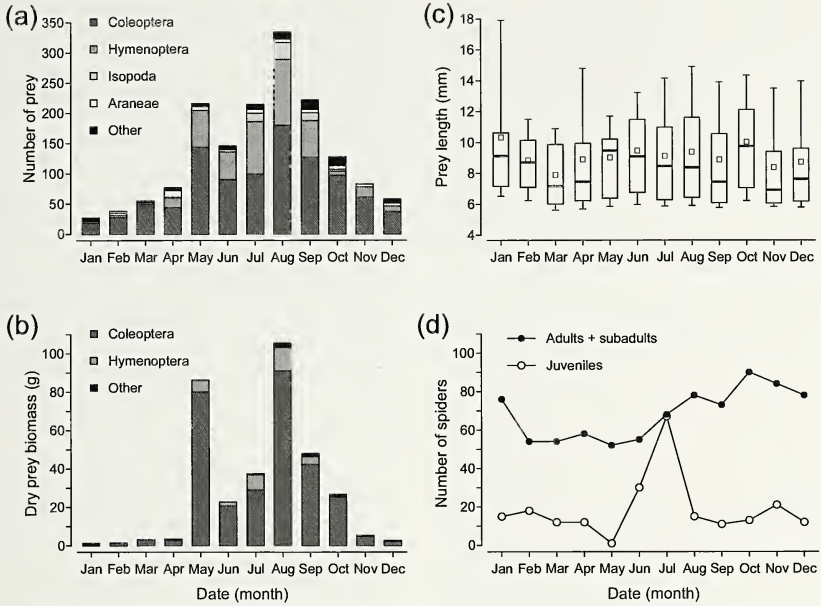

Figure 1.—Prey captured by *Latrodectus hesperus* spiders on a monthly basis in 2005: (a) number of prey; (b) prey biomass (dry); (c) prey length distributions. (d) Number of *L. hesperus* spiders from different age classes present under the sheds. In (a) and (b), prey are grouped according to their taxonomic order with the 4 most abundant orders shown separately and the remainder (Dermaptera, Orthoptera, Lepidoptera and Diptera) grouped into a single category, 'Other'. In (b), only the 2 most abundant orders are shown separately and the remainder is grouped into 'Other'. In (c), box plots show the median (thick lines), mean (open squares), 25th and 75th percentiles (bottom and top of boxes), and 10th and 90th percentiles (cap of lower and upper whiskers); Data for Araneae prey are omitted because they are not based on body length.

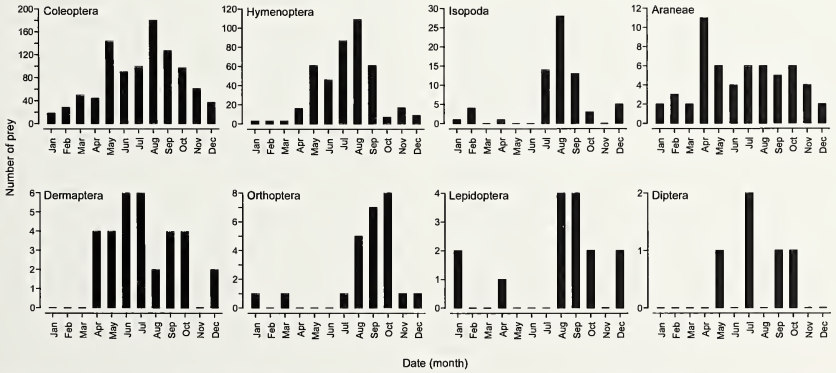

Figure 2.—Number of prey from eight different orders of arthropods consumed by *Latrodectus hesperus* spiders on a monthly basis in 2005. Taxa are presented in order of abundance (left-right).

polyphagous, and insects constitute the largest portion of their diets (Nentwig 1987); other common prey include arthropods such as spiders. However, particular prey taxa are often disproportionate represented in the diets of many polyphagous spider species (see species-specific diet breadth indices in Nyffeler 1999), as was found in this study.

Despite being polyphagous, *L. hesperus* showed a certain degree of dietary specialization on Coleoptera and Hymenoptera, and there was much variation in the prey composition of their diet across different months. It is not known whether this trend reflects habitat-related heterogeneity in prey availability. A study of *L. hesperus* populations in the San Juan Islands, located off the northwest coast of the USA 2 km from my study population, also found that spiders fed mostly on Coleoptera, especially tenebrionid, carabid and scarab beetles (Exline & Hatch 1934). Furthermore, previous research on the diets of other *Latrodectus* species across various habitats has also indicated that the prevalent prey type is Coleoptera. For example, in arid regions of Spain, *L. lilianae* (Melic 2000) feed on a variety of arthropod prey, although predominantly on Coleoptera, which make up the bulk of prey biomass (Hódar & Sánchez-Piñero 2002). Likewise, a foraging study of *L. geometricus* (Koch 1841) living indoors in Brazil revealed a predominance of Coleoptera in their diet among six orders of insects collected from their webs (Rossi & Godoy 2005). Dissections of nests from *L. revivensis* (Shulov 1948) and *L. tredecimguttatus* (Rossi 1790) in Israel and Palestine also showed a predominance of Coleoptera prey remains among several other types of arthropod prey (Shulov 1940, 1948; Shulov & Weissman 1959). Coleoptera are also disproportionately represented in the natural diets of species from other theridiid genera (Riechert & Cady 1983; Nyffeler & Benz 1988). However, *Latrodectus* spiders, including *L. hesperus*, are also important predators of Hymenoptera such as ants and wasps, as shown in this study. In fact, *L. hesperus* may exert a large influence on the activity patterns of ants (MacKay 1982). Examples of *Latrodectus* spiders that feed primarily on ants include *L. pallidus* (Pickard-Cambridge 1872) from Palestine and *L. mactans* (Fabricius 1775) living in cotton fields in Texas, USA (Shulov 1940; Nyffeler et al. 1988).

Conspecifics comprised a small fraction of the diet of *L. hesperus*, despite their facultative web-sharing habits at the study site (Salomon et al. 2010). Like most spiders, *L. hesperus* are opportunistic cannibals that only feed on conspecifics when hungry, when the availability of alternative prey types is low, or following an antagonistic encounter with a conspecific (Mayntz & Toft 2006; Wise 2006; M. Salomon & S. Vibert, unpublished data).

A spider's diet breadth may depend on several factors, including intrinsic factors such as prey-capture behaviour and foraging mode, extrinsic factors such as habitat characteristics and prey ecology, and combinations thereof (Riechert & Luczak 1982; Uetz 1990). Prey-capture behaviour may influence diet breadth in several ways. For example, theridiid spiders such as *L. hesperus* typically capture prey by 'combing' sticky silk around them with their back legs to immobilize the prey (Japyassú & Caires 2008). This foraging technique is thought to be particularly effective at capturing large or potentially harmful prey such as Coleoptera and Hymenoptera (Nentwig 1987). Furthermore, the range of prey sizes captured may also depend on the extent of social interactions during foraging. Species in which individuals forage alone usually capture prey that are smaller or comparable in size, whereas social and partly-social spiders that cooperate during foraging can subdue large prey several times their size (Rypstra 1990; Powers & Avilés 2007). In this study, *L. hesperus* spiders fed on prey that were mostly 50–130% of their adult body size. Based on my many laboratory and field observations of foraging in *L. hesperus*, adults appear to capture and consume prey alone, even when they share webs, whereas juveniles often capture and consume prey as a group, especially large prey. The potent venom and effective prey-capture web of *Latrodectus* spiders may also contribute to the success of some individuals or species at capturing large prey (Forster 1995; Hódar & Sánchez-Piñero 2002). Furthermore, the distribution of prey sizes and taxa in the diet may depend on a spider's prey selectivity associated with particular dietary requirements. Spiders can discriminate between prey based on individual characteristics such as size, external morphology, behaviour and nutrient composition, and thus determine the prey's relative profitability (Riechert & Luczak 1982; Pekár 2004).

Likewise, a spider's foraging mode (i.e., web-based hunting versus cursorial hunting) may determine the ability to forage on a wide versus narrow range of prey types. In a meta-analysis of the diets of spiders living in agro-ecosystems, Nyffeler (1999) found that cursorial spiders generally have a larger diet breadth than web-building spiders. This difference is likely due to the lower accessibility of many prey types by stationary (web-based) versus mobile (cursorial) hunters, although it may concurrently depend on habitat characteristics (see below).

In web-building species, the morphology and location of the web may influence an individual's diet. Web morphology varies both across species and across individuals living in different environments, and a web's structural (e.g., overall geometry, silk thread density) and physical (e.g., position, orientation) characteristics may determine prey-capture rate and prey composition (e.g., Rypstra 1982; Sandoval 1994; Miyashita 1997). Furthermore, some of these web characteristics may represent adaptations for specialized feeding on profitable prey types, thereby narrowing the range of potential prey. For example, the prey-capture component of *L. hesperus* webs consists of sticky 'gumfooted' silk threads that function mostly as trip lines for ground-active arthropods such as Coleoptera and certain Hymenoptera (Blackledge et al. 2005).

Because prey are non-randomly distributed in space and time, the taxonomic composition of prey in a spider's diet largely depends on the location of its web within the habitat (Chacón & Eberhard 1980; Nentwig 1985; Harwood et al. 2001). A spider's actual diet may dependent on local prey diversity and seasonal activity patterns of prey, which determine feeding opportunities (Uetz 1990). By occupying a particular habitat location (either involuntarily or voluntarily) a web-building spider may have access to a specific subset of prey. At the study site in coastal British Columbia, *L. hesperus* spiders live exclusively under driftwood logs (Salomon et al. 2010), which likely restricts opportunities to feed on aerial prey or vegetation-borne prey, and constrains their diet breadth to ground-active arthropods.

The results of this study invite further research on the role of behaviour and life history in the feeding ecology of *L. hesperus*. For example, one could examine whether the diet of *L. hesperus* varies with age, which is likely correlated with prey-capture behaviour and dietary requirements. Based on field observations, I suspect that many of the ants collected as prey were preyed upon by *L. hesperus* juveniles and that subadult and adult females were the ones feeding on wasps. A relationship between predator age and feeding habits may provide insight into important aspects of a predator's biology, such as growth rate and reproductive success.

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Appendix 1.—List of regression equations used to calculate dry prey biomass (y, in mg) based on total body length (x, in mm) for different orders of arthropods. For Araneae prey, the calculations were based on tibia-patella length of leg pair I (tp, in mm) and wet prey biomass (w, in mg)

Prey taxon	Regression equation	R	R^2	Source
Coleoptera	$\ln(y) = -3.460 + 2.790 \ln(x)$	0.98	–	Rogers et al. 1977
Hymenoptera	$\ln(y) = -3.871 + 2.407 \ln(x)$	0.97	–	Rogers et al. 1977
Isopoda	$y = 0.010\ x^{2.844}$	–	0.96	Hódar 1996
Dermaptera	$y = 0.002\ x^{3.497}$	–	0.96	Hódar 1996
Orthoptera	$\ln(y) = -3.020 + 2.515 \ln(x)$	0.97	–	Rogers et al. 1977
Lepidoptera	$\ln(y) = -4.037 + 2.903 \ln(x)$	0.99	–	Rogers et al. 1977
Diptera	$\ln(y) = -3.293 + 2.366 \ln(x)$	0.96	–	Rogers et al. 1977
Araneae				This study
Latrodectus hesperus:	$\ln(w) = 1.948 + 2.032 \ln(tp)$ ($P < 0.0001$, $n = 86$)	–	0.23	
	$\ln(y) = -1.846 + 1.132 \ln(w)$ ($P < 0.0001$, $n = 32$)	–	0.92	
Tegenaria agrestis & T. duellica:	$\ln(w) = 3.038 + 1.253 \ln(tp)$ ($P = 0.007$, $n = 28$)	–	0.22	
	$\ln(y) = -1.745 + 1.100 \ln(w)$ ($P < 0.0001$, $n = 16$)	–	0.87	
Lycosidae:	$\ln(y) = -0.679 + 2.643 \ln(tp)$ ($P < 0.0001$, $n = 32$)	–	0.65	

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Contrasting energetic costs of courtship signaling in two wolf spiders having divergent courtship behaviors

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Abstract. Energetic costs of courtship behavior were measured for two sympatric wolf spiders that are reproductively isolated based on distinct male courtship behaviors with different signaling modes and activity levels: *Schizocosa ocreata* (Hentz 1844) uses multi-modal communication (visual and seismic signals) and an actively-moving courtship display, whereas *S. rovneri* (Uetz & Dondale 1979) uses only seismic signals produced while stationary. To test for increased energetic expense of more complex multimodal courtship in *S. ocreata*, we recorded peak CO_2 output for male spiders standing, walking, or courting. We found that peak CO_2 output while standing or walking was similar between species. Courtship behavior of *S. ocreata* produced greater peak CO_2 output than these other behaviors, and was significantly greater than peak CO_2 output of *S. rovneri* courtship, which was not different from that of locomotion. Hence, unequal energy expenditure related to the modality of the males' courtship displays resulted in different energetic costs for courting male spiders. Male courtship vigor may serve as a criterion for female mate choice in *Schizocosa*.

Keywords: Courtship, energetics, *Schizocosa*, respiration, sexual selection

INTRODUCTION

Differences in male courtship displays between spider species may serve as behavioral isolating mechanisms for closely-related taxa (Stratton & Uetz 1981, 1986; Miller et al. 1998; Stratton 2005), but may also reflect the influence of sexual selection based in part on differential energetic costs (Kotiaho et al. 1998; Parri et al. 2002; Delaney et al. 2007; Byers et al. 2010). Much support for "handicap" or "good genes" models of sexual selection suggests that females prefer males capable of sustaining higher levels of energetically costly motor performance (see review by Byers et al. 2010), because active, complex courtship display behaviors provide "honest" information to females about male condition or quality (e.g., Zahavi 1975; Zuk 1991; Andersson 1994; Kotiaho et al. 1996, 1998). For example, in the well-studied European wolf spider *Hygrolycosa rubrofasciata* (Ohlert 1865), females choose males on the basis of drumming rates, which are good predictors of male condition and viability (Kotiaho et al. 1996; Kotiaho et al. 1998; Kotiaho 2000; Ahtianen et al. 2005, 2006).

Wolf spiders (Lycosidae) use active courtship displays and multimodal communication (visual and seismic cues) to varying degrees (Kotiaho et al. 1998; Hebets & Uetz 1999; Hebets et al. 2006; Uetz & Roberts 2002; Uetz et al. 2009). Within the genus *Schizocosa*, the *S. ocreata* clade contains 6–8 species that apparently have arisen via behavioral isolation driven by sexual selection (Miller et al. 1998; Stratton 2005). Members of this clade are similar in size and coloration, have nearly identical genitalia, and females are largely indistinguishable. Males, however, vary in the degree of decoration of their forelegs (ranging from little or no pigmentation to dark pigment and tufts of bristles) and courtship behavior (stationary vs. active movement; unimodal vs. multimodal signals) (Stratton & Uetz 1981, 1986; Hebets & Uetz 2000; Uetz & Roberts 2002; Stratton 2005). While energetic costs of courtship signaling are currently unknown for *Schizocosa*, several studies suggest that highly active multimodal signaling may be more costly (Delaney et al. 2007; Roberts et al. 2007; Uetz et al. 2009). In this study, we test this hypothesis by examining the energetic costs of courtship display for two well-studied sibling species: *Schizocosa ocreata* (Hentz 1844) and *S. rovneri* (Uetz & Dondale 1979). Given the observed active, multimodal courtship of *S. ocreata* versus the more stationary, unimodal courtship of *S. rovneri* (Delaney et al. 2007; Uetz et al. 2009), we predicted that *S. ocreata* will incur higher energetic costs than its sibling species.

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METHODS

Study species.—The brush-legged wolf spider, *Schizocosa ocreata*, and its sympatric sibling species, *S. rovneri* are often referred to as "ethospecies", because while physically capable of interbreeding (Stratton & Uetz 1986), the species remain isolated due to distinct communication behaviors permitting pre-mating species recognition and reproductive isolation (Stratton & Uetz 1981, 1986). Male *S. ocreata* possess dark pigmentation and conspicuous tufts of bristles on the forelegs used in visual courtship displays while these tufts and visual displays are lacking in *S. rovneri*. Males court conspecific and heterospecific females and their silk with equal frequency (Roberts & Uetz 2004), but females only mate with conspecifics (Uetz & Denterlein 1979; Stratton & Uetz 1981). Despite the highly effective behavioral barrier, these species are highly similar at the molecular phylogenetic level (Hebets & Vink 2007), potentially interfertile, and capable of producing interspecific hybrids (Stratton & Uetz 1981, 1986; Orr & Uetz unpubl.), suggesting a relatively recent evolutionary divergence (Stratton 2005).

Courtship display behaviors differ considerably between these two species (Delaney et al. 2007; Uetz et al. 2009). The courtship of male *S. rovneri* consists predominately of a single display performed while stationary. The body "bounce" combines substratum-coupled stridulation (rotation of pedipalps) and percussion (the body, abdomen and/or chelicerae

sometimes strike the substratum). A "leg extend" display is also occasionally produced. In contrast, the courtship display of *S. ocreata* is far more active, and consists of two displays performed during locomotion ("double tap" and "jerky tap") and two while stationary ("leg extend" and "wave/arch"). Seismic signals from stridulation (pedipalps) and percussion (abdomen and chelicerae striking the substratum) are produced simultaneously with visual signals during the "jerky tap" display. Analyses here were centered around three main behaviors: stationary (the spiders remains motionless), locomotion (the spider walks, explores, or otherwise moves around), and courtship (specific courtship behaviors displayed as described above).

Animal maintenance.—We collected immature spiders in April–May 1997 from sites containing only one of the two species: *S. ocreata* from the Rowe Woods facility of the Cincinnati Nature Center, Clermont Co., Ohio, and *S. rovneri* from Sandy Run Creek, Boone Co., Kentucky. We maintained all spiders individually in the laboratory until sexual maturity in opaque plastic containers (10 cm diam.) under identical controlled conditions (22–24° C; light:dark cycle = 13:11 h). All spiders received water ad libitum and 4–5, 10-day old live crickets (*Acheta domestica*) as food once/week.

Measurement of energetic output.—We collected data on CO_2-production as a function of male behavior using a Sable Systems TR-2 flow-through respirometry system. A multiplexer controlled flow of CO_2-free air (75 ml/ min) and gas mixtures throughout the purging and data collection segments of each trial. We monitored temperature continuously throughout test runs using integrated thermocouples. Data acquisition, integration, and initial analyses used Sable Systems software (Sable Systems, Salt Lake City, Utah). Data were acquired from the test chambers and data logger at one-second intervals.

We first placed each of 13 male *Schizocosa* spiders into a 25 ml, cylindrical, clear acrylic test chamber with stoppers fitted with tube couplings and valves at each end. The animal acclimated at least 10 min in the chamber while chamber temperature stabilized. Immediately before testing, we purged the chamber to create a standard air environment of 15-ppm CO_2. After purging and standardization, we attached the chamber to the respirometer and the trial began.

Each 20 min trial consisted of sequentially logging non-courtship behavior followed by courtship using two 10-min periods of collecting, observing, and logging behaviors displayed by an individual spider using the integrated behavior logging feature of the software. The first 10 min provided baseline measurements of CO_2 liberation during stationary and locomotory behaviors. After the initial 10 min, we introduced a piece of paper (~1 × 3 cm) cut from the substrate ("cage card") of a female conspecific *Schizocosa* into the test chamber with the male. This paper held chemical cues triggering courtship in the male (Roberts & Uetz 2004 a,b; Roberts & Uetz 2005). We purged the chamber and again placed the spider into the respirometer for 10 min to monitor and log courtship behaviors as above.

We adjusted measurements of liberated carbon dioxide relative to spider mass, temperature, and observed duration of behaviors via the Sable software and graphed the results (Fig. 1). We extracted values for observed peaks of CO_2 output, (µl/g/h) during selected periods of three main behaviors: stationary, locomotion, and courtship, which then served as the bases for analyses. We determined the peaks associated with these behaviors by visually inspecting the respirometer output of lagged synchrony with time-stamped event recording (see Fig. 1).

We recorded multiple peak CO_2 values for each behavioral category for seven *S. ocreata* and six *S. rovneri* males (Leger & Didrichsons 1994), and analyzed for interspecific differences in peak values using the Mann-Whitney U-test. We also calculated means ± SEM for each of the six data categories in order to calculate the ratios of energetic output.

RESULTS

During the first observation period, all males (n = 13) alternated bouts of locomotor activity with periods of stationary resting behavior (Fig. 1). They all exhibited courtship behavior during the second observation period (after purging the chamber) upon contacting the paper substrate containing silk from conspecific females.

As expected, locomoting spiders produced much higher peak CO_2 output relative to stationary ones: *S. rovneri* = 120.6%; *S. ocreata* = 107.7% (Fig. 2). Furthermore, courting males were even more active than when they were at rest: *S. rovneri* = 153.2%; *S. ocreata* = 225.4% (Fig. 2).

Analyses of peak CO_2 output revealed no differences between species for stationary or locomotor behaviors (Fig. 2). In contrast, courting *S. ocreata* males had a significantly greater peak CO_2 output than *S. rovneri* males (U = 560, P= 0.022; Fig. 2). The more active courtship of *S. ocreata*, comprised of a "jerky-tap" display which includes forward locomotion, leg-tapping, and leg-waving, produced a 36.6% higher level of peak CO_2 output than *S. rovneri.* Additionally, the degree of difference between levels of peak CO_2 output during courtship and locomotor activity for *S. ocreata* was much greater (56.6%) than that for *S. rovneri* (14.7%), who remain stationary during courtship. Thus, the rate of increase for energetic costs for the behavioral transition from a stationary state to active courtship is greater for *S. ocreata* than *S. rovneri.*

DISCUSSION

Our results show that, while stationary and resting metabolism of both spider species are similar, courtship is more energetically expensive for *Schizocosa ocreata* than it is for *S. rovneri* males. The multi-modal signaling of *S. ocreata* (with visual and seismic components) likely accounts for a greater difference in resting versus courtship CO_2 liberation compared to that of the stationary unimodal display of *S. rovneri* (seismic only). Hence, differences in CO_2 output during courtship between these species supports our initial hypothesis.

Using peak CO_2 values as a metric of energetic output by spiders is complicated because a proportion of the expired CO_2 could originate from hemolymph bicarbonate due to lactate production (Prestwich 1983, 1988a,b). Lactate reaches maximum concentration approximately 10 min after vigorous exercise in theraphosid spiders (i.e. tarantulas on treadmills, Paul & Storz 1987). In lycosids, depending on intensity of activity, lactate may reach very high levels in 30 s, or it may

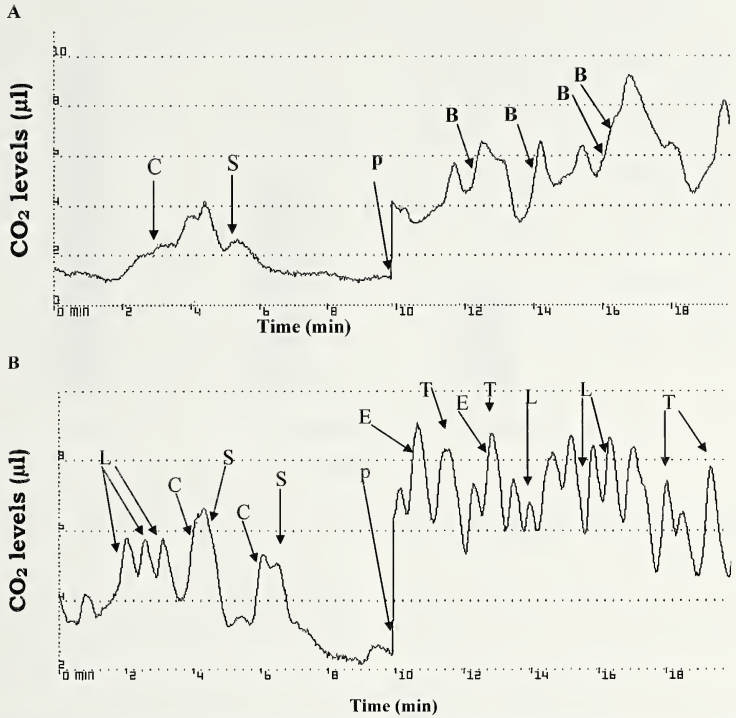

Figure 1.—Representative CO_2 output profiles for male *Schizocosa* during the two observation periods (first period = 10 min resting/walking; second period = 10 min active courtship after stimulation of chemical cues from silk of conspecific female): **A**. Male *S. rovneri*; **B**. Male *S. ocreata*. Bouts of different behavioral activities are indicated by arrows on the graph (note different ordinate scales for A and B). Abbreviations: B = bounce (the spider strikes the substrate with the sternum); C = climb (moving up the chamber's side and supporting the body on rear legs); E = explore (using forelegs to probe the area ahead or below the spider); L = locomotion (walking or generally moving around the chamber); p = purge test chamber after addition of female silk; S =stridulate (the spider places tips of palpal tarsus on substrate and flexes stridulatory organ between palpal tibia and tarsus); T = tap (simultaneous raising of forelegs prior to simultaneously striking the substrate with both legs).

not accumulate significantly even after longer activities (Prestwich pers. comm). Thus, the influence of lactate on $VDCO_2$ (= the volume of CO_2 produced per unit time) may complicate the comparison of different activities in dissimilar species.

Our study compares very similar species performing similar types of activities. Our analyses used $VDCO_2$ recorded only at peaks of activity for the three basic behaviors, and possibly overestimates aerobic metabolism during these activities. However, because we compared very similar behaviors in sibling species, overestimates are likely to be parallel, and useful comparisons are still possible. In fact, the potential overestimate of aerobic metabolism obtained by using $VDCO_2$ in these specific cases is advantageous because it qualitatively accounts for any anaerobic metabolism contributing to the total cost of the activity (Prestwich pers. comm.). Measurements of VDO_2 alone would not do this. Thus, in these limited comparisons, $VDCO_2$ should represent a reasonable metric of comparison.

There are two non-exclusive hypotheses that might explain observed differences in the energetic expense of courtship behavior between these species. For example, differences may reflect the influence of environmental constraints on signaling. Attenuation of seismic courtship signals in leaf litter microhabitats has been suggested as the reason *S. ocreata* uses multi-modal signaling incorporating simultaneous visual signals (active leg-waving and tapping) along with production of seismic signals by stridulation and percussion (Scheffer et al.1996; Uetz 2000; Uetz & Roberts 2002; Uetz et al. 2009). In addition, multiple substratum types (leaves, bark, twigs, soil, rocks) within the complex litter habitat vary in capacity to convey seismic signals (Elias et al. 2004; Hebets et al. 2008; Elias et al. 2010; Gordon & Uetz, in review). Thus, *S. ocreata* courtship displays must include more overt visual components

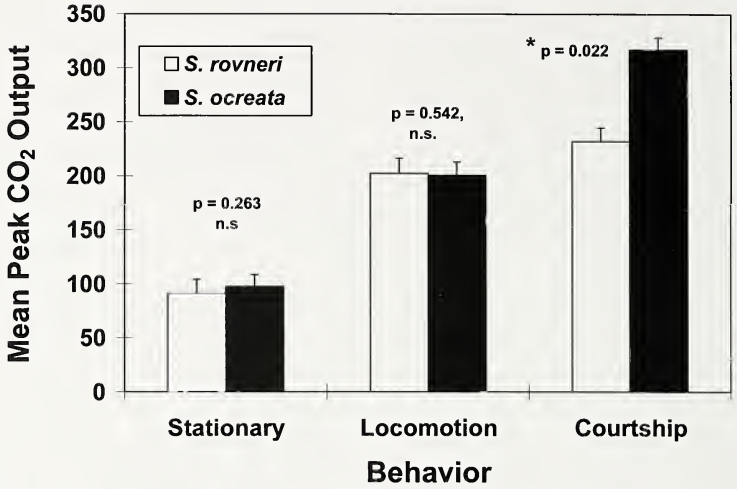

Figure 2.—Mean (± SEM) peak CO_2 output (µl/g/hr) for male *Schizocosa ocreata* (n = 7) and *Schizocosa rovneri* (n = 6) during bouts of stationary, locomotor, and courtship behavior. Results of pairwise statistical comparisons (Mann-Whitney U-test) between species are indicated. Abbreviations: NS = not significant; * = significant at $P < 0.05$.

(Scheffer et al. 1996; Uetz et al. 2009), which demand greater energy expenditure. The compacted litter substrate of *S. rovneri* transmits seismic vibrations up to 50 cm (Scheffer et al. 1996), allowing use of a less energetically-demanding percussive "body bounce", to convey signals on this surface.

Differences in courtship vigor also could reflect sexual selection for performance in male signaling, as vigorous courtship display may serve as an "honest indicator" of male condition on multiple levels (Zahavi 1975; Zuk 1991; Kotiaho 2000; reviewed in Byers et al. 2010). For example, highly-active males of the drumming wolf spider *Hygrolycosa rubrofasciata* incur greater energetic expense, but are preferred as mates and produce offspring with higher survival rates than those males displaying less drumming (Mappes et al. 1996; Kotiaho et al. 1996, 1998; Kotiaho 2000; Parri et al. 2002). However, male *H. rubrofasciata* with higher drumming rates also suffer reduced immune function (Ahtianen et al. 2005, 2006). Likewise, males of both *S. ocreata* and *S. rovneri* exhibiting higher signaling rates have greater mating success (Delaney 1997; Delaney et al. 2007; Gibson & Uetz 2008). At the same time, the increased conspicuousness of vigorous male *S. ocreata* signaling may increase detection by visual predators, whereas *S. rovneri* may not (Pruden & Uetz 2004; Roberts et al. 2007). Thus, if signaling traits are indicators of a male's ability to assimilate, store, and use energy, or indicate higher levels of immune function or viability, a female receiving gametes from these males would obtain genes conferring superior foraging, metabolic and/or immune response abilities for her offspring.

In conclusion, while the active multimodal signaling of *S. ocreata* undoubtedly contributes to increased efficacy of communication within complex environments that constrain particular channels of communication (Scheffer et al. 1996; Hebets & Papaj 2005), that increased efficacy comes with a higher energetic cost (current study) as well as increased predation risk (Pruden & Uetz 2004; Roberts et al. 2007). Consequently, the energetic expense associated with complex signals used by male *S. ocreata* would therefore represent the basis for multimodal courtship as an "honest indicator" of male quality or condition (Zahavi 1975; Zuk 1991; Ketola & Kotiaho 2009; Byers et al. 2010), and provide indirect fitness benefits as a criterion for female mate choice.

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BOOK REVIEW

Scorpions of the World. By Roland Stockmann and Eric Ythier. 2010. N.A.P. Editions, France. 567 pp. ISBN 978-2-913688-11-7. 75€.

An old Egyptian proverb cautions "Because we focused on the snake, we missed the scorpion." For years this proverb has characterized the status of reference books on snakes and scorpions, as comprehensive sources containing knowledge on both the biology and diversity of scorpions were sorely lacking – that is until now! In their new book *Scorpions of the World*, French biologists Roland Stockmann and Eric Ythier present for the first time a guide to the biology and biodiversity of the world's extraordinary scorpions. Published in both English and French (*Scorpions du Monde*), the book is organized into six main sections with a handy list of species and their distributions, as well as a glossary. Exquisite illustrations and scanning electron micrographs are found throughout, and color plates accompany over 350 species descriptions, many of which describe species that are rare or difficult to find. The book is bound in a beautiful hard cover that exhibits a striking photo of an adult *Hadogenes paudicens* with an instar on its carapace. Inside the front and back covers one will find a table of contents and illustrations of *Androctonus australis* labeling the general external anatomy of a scorpion. One of the best features of this book is its size, only slightly larger than a typical field guide and small enough to be carried into the field by adventurous scorpion collectors. While not an exhaustive summary of the world's scorpions, many of which have yet to be discovered, the book should prove useful for identifying many of the scorpion species most commonly encountered in collections and in the field.

The book begins with a short but elegant foreword by Victor Fet, editor of *Euscorpius* (a peer-reviewed journal dedicated to scorpions), and one of the most active researchers in the field. Next, a substantial introductory section, conveniently organized into multiple subsections, focuses on general topics such as paleontology, general morphology, classification criteria, and collection and preservation techniques of scorpions. The section on classification criteria is incredibly useful as it provides a single up-to-date reference for many of the intricate characters used in current classification schemes; characters such as coloration, trichobothria positions, spermatophore and ovariuterus details, and many variations in external morphology. I have already found myself reaching for the book and opening to this section to look up the sometimes confusing nomenclature of fine-scale anatomical features like carinae and trichobothria. Using these characters and DNA sequence data, researchers, using cladistics, have proposed two different suprageneric level classifications, both of which have been fiercely debated (Soleglad & Fet 2003; Fet & Soleglad 2005; Prendini & Wheeler 2005). I am happy to see that both of these classifications, with slight modifications, are presented in the book.

The next section is just as detailed as the first and contains an abundance of information on anatomy, venoms, defensins, and biological functions, topped off with a small dose of behavior and ecology. Portions about venoms, defensins, and blood are written by Max Goyffon and provide a detailed introduction to these topics. These sections, however, are a bit more in depth than the rest of the volume, slightly deviating from the authors' intention to write a book for amateur naturalists and not for specialist arachnologists. Nevertheless, the writing is superb, and Goyffon's outline of defensins and the architectural similarity of defensin and venom peptides was especially thought provoking, especially from an evolutionary standpoint. From there the book outlines the nervous system and sensory organs such as eyes, setae, and various chemoreceptors. Quick to reference renowned naturalist Jean-Henri Fabre, who hailed from their own country, the authors also provide a thorough description and illustrations of scorpion courtship and all the intricate behaviors that can be involved, followed by notes on embryology, parturition, parthenogenesis, growth, and molting. The section on ecology begins with my favorite illustration of the book, a common burrow of *Heterometrus fulvipes* that resembles a cross section of a human heart, only with various-sized scorpions crawling out different branches of the aorta! Predator-prey relationships are briefly discussed, as well as theories about r/K selection strategies. Scorpion ecotypes, from psammophiles to troglobites, are explained, and burrows and digging behavior are outlined alongside a figure of assorted burrow types.

The book then progresses to a discussion about the capability of scorpions to endure various environmental stresses such as desiccation, extreme temperatures, starvation, and fire cycles (by remaining protected in their burrows). Goyffon then takes over the writing again, this time steering the book in a strange but interesting direction, exploring the resistance of scorpions to ionizing radiation. While it is relatively well-known that scorpions and beetles are among the only animals known to survive near nuclear testing areas, it is seldom mentioned that a bit of research has been done on the subject. In 1963, the French government founded a laboratory in Paris at the Muséum National d'Histoire Naturelle with the purpose of studying the radioresistance of scorpions. Research in the lab ceased 10 years later, but some interesting results from the unique studies that took place there are presented in the book with some detail. Goyffon continues with the next section on envenomations as well, in

which he provides tables listing dangerous species and outlines symptoms and treatments for human envenomations.

Unlike previous works on the biology of scorpions, a section on scorpion husbandry is also provided, undoubtedly authored by Ythier, who has kept numerous species from all over the world. The section is short but accompanied by a convenient two-page table of ideal rearing conditions for 46 genera from eight families. Nine pages are subsequently devoted to myths, legends, and representations of scorpions in ancient writings, art, science, and pop culture. Finishing up the textual portion of the book is a section on taxonomy with a detailed dichotomous key that should be useful for identifying any scorpion at least to family level. Tables listing the genera and number of species therein are also provided for each family.

The latter half of the book is printed in color on glossy white pages. It begins with several pages of plates referred to in the text, and is followed by a section on biotopes with color pictures of a variety of specific habitat types associated with various species. A sand desert in Morocco, for instance, complete with palm trees silhouetted against the desert sun represents habitat for the aggressive species *Buthacus arenicola*. In stark contrast, a lush tropical rainforest is indicated to house the Brazilian scorpion *Tityus costatus*.

Species descriptions comprise most of the remaining pages. Over 350 scorpion species are presented in color with pictures taken by 29 photographers from around the globe. Each description contains about a paragraph of information on characters useful in identification. Most of the characters chosen are visible to the human eye, making some scorpion identifications much easier for amateur naturalists and researchers working in the field. Venom toxicity, based on taxonomically guided extrapolations of venom studies on a handful of species, is ranked on a scale of one to four both in the text and by small scorpion pictograms shaded white, gray, black or red, with red reserved for only the most venomous species. Habitat is also briefly described for each species, and the degree of preferred aridity is indicated by pictograms of a sun, sun and clouds, or clouds with rain; signifying xeric, mesic, and humid environments respectively. Species descriptions are arranged by seven color-coded geographic regions: North America, Central America and the Caribbean, South America, Europe, Africa, Asia and the Middle East, Australia and Oceania. Distribution maps also accompany each species description, although in some cases the precision is limited because the distributions are restricted to the extent of the countries where the species have been documented. This becomes a problem for the larger countries such as the United States, Brazil, Australia, and China where scorpion distributions are often actually only small areas within them. In addition, I did notice one species that was misidentified (an immature *Smeringurus vachoni* is portrayed next to the description of *Serradigitus joshuaensis*), a pardonable mistake considering the worldwide scope of this book. Some of the venom toxicity rankings were questionable as well, although any system for ranking venom will be somewhat subjective.

Nevertheless, the species descriptions section of the book is a joy to browse and works as an excellent quick reference for those interested in the general appearance, description, venom toxicity and habitat of many species. While descriptions of all the nearly 1,900 scorpion species in the world were far beyond the scope of the book, a useful list of these species, as well as their general distributions, are listed at the end.

After reading this book I realized that while it sheds light on well-studied subjects of scorpion biology and diversity, the lack of information on other subjects highlights areas of research that still need to be investigated. Ecology, for example, is surprisingly scant, especially when one considers that scorpions represent an ideal model organism for many ecological studies. Biogeography and molecular systematics, subjects in which I am currently developing my own research program, are hardly mentioned. The section on scorpion parasites is unfortunately limited to a single paragraph, perhaps owing to the short supply of research on this topic.

Despite just a few shortcomings, this book is well worth the price. In fact, this one-of-a-kind book should prove to be an indispensible reference on scorpions, joining the ranks of *The Biology of Scorpions* (Polis 1990) and *Catalog of the Scorpions of the World (1758–1998)* (Fet et al. 2000). This beautifully crafted compendium is sure to inspire young future scorpiologists. *Scorpions of the World* belongs on the bookshelf of every serious scorpion enthusiast, and in public and university libraries around the world so that others can discover the incredible diversity in one of the world's most notorious animal groups.

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SHORT COMMUNICATION

Cannibalism within nests of the crab spider *Misumena vatia*

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Abstract. About 1% of the nests of a crab spider (*Misumena vatia* [Clerck 1757]) population in coastal Maine, USA, contained apparently cannibalistic individuals. These spiderlings remained in their nests over three times longer than average and attained average masses twice that of non-cannibalistic spiderlings (maximum = four-fold) before dispersing. Parents of the 14 cannibalistic broods came from 10 sites separated from each other by 0.5–10 km and over 23 years; thus, this behavior appears to be widespread and relatively stable, though uncommon.

Keywords: Fitness, local population, Maine, Thomisidae

Recently, cannibalism among just-born young, especially as it relates to possible kin selection (Pfennig 1997; Roberts et al. 2003; Morse 2011), has attracted considerable attention. Many spiders molt into a fully active form within a protective nest or egg sac. However, proclivity toward cannibalism does not seem to have been addressed in offspring prior to emergence from these sites, a period that involves potential interactions only with kin. Under these circumstances, victims of cannibalism would function analogously to trophic eggs (Crespi 1992), enhancing the fortunes of some sibs at the expense of others. Observers could easily miss cannibalism at this point, because it normally would be hidden from view. Here I present evidence from second-instar crab spiders *Misumena vatia* (Clerck 1757) (Thomisidae) strongly suggesting that cannibalism takes place within the nests of a very small minority of broods prior to their emergence.

In the process of obtaining data on several early life history parameters (Morse & Stephens 1996), I collected large adult female *M. vatia* from flowers in fields and roadsides at 18 sites in South Bristol, Bristol, and Bremen, Lincoln Co., Maine (centering on 43°57′N, 69°33′W) during June and July 1987–2009. Adult females of these populations whose mass has increased considerably since molting have almost inevitably mated (LeGrand & Morse 2000). I maintained these individuals in 7-dram vials (5 cm tall, 3 cm diameter) and fed them moths or other insects every other day until they reached a mass at which they would normally lay a clutch of eggs if in the field. I then placed these gravid females on non-flowering common milkweed *Asclepias syriaca* ramets, a favored oviposition site, within bags of white nylon tricot (30 cm tall, 20 cm wide) that confined them to the site, but provided adequate space for them to construct their nests on the distal parts of leaves. Nest building consists of turning under the tip of a leaf, laying eggs within the resulting chamber, filling the remainder of the chamber with flocculent silk and tightly securing the top, bottom and sides with silk to produce the finished nest (Morse 1985). After they laid their eggs and completed their nests, I removed the bags from the milkweed ramets. Subsequently, I visited the nest sites daily to document their status and that of the guarding females (described in Morse 1985, 2007).

During the 1987–1989 seasons I processed especially large numbers of female *M. vatia* (over 200/year) in order to obtain detailed information on several reproductive and developmental parameters. Beginning at 20 days after egg laying I carefully inspected the nests each day for openings in the silk produced by the young, which would usually lead to their departure from the site a few days later (see below). Prior to leaving the nest, spiderlings frequently occupied the entrances of these openings or the nest surface immediately outside them.

In the process of these observations I discovered a small number of nests in which the young did not all depart within a few days of the initial openings. I subsequently noted that individuals at the surface of these openings appeared larger than usual, so I collected, counted and weighed these young and recorded how many days any of them remained in their nests. I compared the prelaying mass of the mothers of the lingering broods, most of which had died or abandoned their nests by this time (Morse 1987), with those from the other nests with similar initial emergence dates, 11–19 August, to control for possible variation resulting from seasonal changes in temperature. I also had available measures of spiderling mass at dispersal (Morse 1993a) and prelaying mass of mothers (Morse 1985, 1987, 2009; Morse & Stephens 1996) from other studies on these populations, which allowed additional comparison.

In addition to the 1987–1989 data, I processed similar broods during 13 subsequent seasons (1990–2000, 2008–2009). Use of the broods in these years did not allow me to obtain some of the supporting data gathered in 1987–1989, thus precluding direct comparisons. During most of these years I reared between 40 and 80 reproductive females. (From 2001 to 2007 I used reproductive females for experiments that did not permit me to obtain any of these data.)

Comparisons between the two types of broods, lingering or directly dispersing, were tested for significance with two-way *t*-tests for the difference between two means. All measures of variance are means ± 1 SE.

Numbers of nests with large, lingering spiderlings constituted only a minute fraction of the nests that I monitored over this period -1987: 2 of 227 (0.9%); 1988: 3 of 280 (1.1%); 1989: 2 of 271 (0.7%); combined, less than 1% of all nests (Table 1). I probably would not have discovered these individuals without the prodigious effort made during these years to obtain other data (presented elsewhere).

In the nests where spiderlings lingered, only one to five remained at the nests after young had departed from most nests (Table 1). Spiderlings in these nests weighed, on average, twice as much as normally dispersing young (0.6 mg average mass), with a maximum-sized individual (2.33 mg) four times as great. In some instances the differences in mass even suggested that they had preyed on specific numbers of sibs; for instance, one set of remaining young weighed 0.97, 1.25, 1.43 and 1.52 mg (probably one, two, three, and four young, respectively). Other than for their large mass, individuals from these broods did not appear to differ morphologically from spiderlings of the other broods.

These spiderlings have no apparent source of sustenance in the nests or at the entrances to these nests other than their sibs. I have never observed spiderlings feeding on insect prey at the entrances to

Table 1.—Characteristics of putative cannibalistic and non-cannibalistic spiderlings (mean ± SE), with *n*'s in parentheses.

Trait	Cannibals	Non-cannibals	*df*	*t*	*P*
Number in nest	2.8 ± 0.54 (7)	119–368‡ (49)	–	–	
Mass at dispersal (mg)	1.2 ± 0.05 (17)	0.6 ± 0.04 (30)	45	9.25	< 0.0001
Time at nest (days)	17.3 ± 1.76* (7)	5.3 ± 0.36 (41)	46	12.56	< 0.0001
% nests	0.9 (778)	99.1 (778)	–	–	–
Maximum mass of mother (mg)	216.6 ± 17.08 (7)	209.5 ± 3.15 (206)	211	0.41	0.69

* An underestimate because field season ended before all young left two of the nests.

‡ Number of young per nest in these results not measured. Estimate from comparable source (Fritz & Morse 1985).

these openings, either during 1987–1989 or at other times. Their mothers typically place nests a considerable distance away from sites that would attract the small prey upon which they will eventually feed (Morse 1993b). Further, I did not locate any of these experimental sites close to flowers that would attract potential prey.

Upon dissection, the nests contained several corpses (not counted), which were readily distinguishable from the molts of these individuals. Success of eggs is normally extremely high in these nests (94.5%: Fritz & Morse 1985), with the majority of unsuccessful individuals recorded as unhatched eggs, so that few corpses occur in most nests. Judging from the number of molts found in these seven nests, substantial numbers of sibs probably escaped from the nest. However, I have no information on their traits.

The mothers of these putatively cannibalistic broods (henceforth = cannibalistic) did not significantly differ in size from the mothers of the other broods (Table 1) and thus probably did not differ in condition from them. I obtained no other information on the mothers of the cannibals that would separate them from the other females.

Mothers of the seven broods from 1987–1989 came from five sites, which were separated from each other by 0.5 to 10 km. I did not obtain cannibalistic broods from any of these sites in more than one of these three years. Five of the cannibalistic broods hailed from the largest collection sites of females (and presumably the largest populations as well). The other two broods came from the seventh and eighth largest of 18 collection sites. Between 1987 and 1989 minimum yearly counts of reproductive females at the five sites yielding parents of cannibalistic broods ranged from 15 to 127 (48 ± 20.8), and minimum counts of reproductive females at sites not yielding such broods ranged from 1 to 51 (12 ± 3.8) ($t_{16} = 3.08$, $P = 0.007$).

I recorded seven additional cannibalistic broods during 1990–2000 and 2008–2009. Two of these broods came from the same sites as 1987–1989, and the other five came from different sites. Thus, I obtained females with cannibalistic broods from 10 sites. All of the 10 sites yielding the mothers of cannibalistic broods are separated by a minimum of 0.5 km. The records obtained after 1987–1989 suffice to indicate that this trait continues to occur at low frequency in local populations, and to suggest that this frequency has remained relatively constant over time.

It is unclear how often cannibalism occurs among populations of spiders and other organisms at this early developmental stage because of the difficulty of recording under most circumstances. The origin of this trait is also unclear; although it might appear to have a genetic basis in light of its steady recurrence at a low frequency, I have no direct evidence for this hypothesis.

These cannibalistic broods stand in stark contrast to the vast majority of *M. vatia* broods, which show extreme reluctance to cannibalize either brood mates or members of other broods (Morse 2011). Other workers have reported intrapopulation differences in the propensity of early-instar spiderlings to cannibalize. In particular, Hvam et al. (2005) and Mayntz & Toft (2006) propose the presence of cannibalistic morphs in two different species of *Pardosa* wolf spiders, although they do not provide information to verify whether these traits have a genetic or environmental basis. I know of no such studies that have explored this trait within the nest or egg sac.

The cannibals' mothers do not differ from other females in any parameter I have measured (Morse 1985, 1987, 2009; Morse and Stephens 1996). The collection sites of the cannibals' mothers are scattered through a region that consists primarily of forest and water, unfavorable sites for *M. vatia*, such that only limited gene flow probably occurs, even though the spiderlings balloon readily (Morse 1993a, 2005). Thus, this cannibalistic predisposition is most likely widespread and not the property of a single large regional population.

The pattern seen in *M. vatia* obviously bears considerable resemblance to the cannibalistic morphs in a wide variety of taxa, including some salamanders and fish. Although many of these individuals show striking morphological variation (e.g., Nyman et al. 1993; Michimae & Wakahara 2002; Klemetsen et al. 2003), others do not exhibit morphological variation (e.g., Lanoo et al. 1989). In these instances, cannibalism is presumably an adaptation to temporary and unpredictable conditions, and perhaps most closely resembles the condition seen in *M. vatia*.

Certain spiders (Gundermann et al. 1991) produce trophic eggs, in common with several other groups (Crespi 1992). Others feed on eggs inside the egg sacs or nests (reviewed in Valerio 1974). Although most of these instances relate to egg feeding by first instars, Valerio (1974) reports instances of active second-instar theridiids feeding on eggs, which suggests the feasibility of sib cannibalism (second instars) within the egg sac or nest. The production of relatively small numbers of large young is adaptive under some circumstances (Roff 1992; Stearns 1992), though cannibalism seems an inefficient way of accomplishing such an advantage. Emergence sizes of non-cannibalistic *M. vatia* broods already vary by nearly two-fold (ca 0.4–0.7), a difference that appears to have a genetic basis (Fritz & Morse 1985), so considerable variation exists for selection to act on these populations. However, this variation does not match the size range of the cannibalistic spiderlings in this study, on average double the size of non-cannibalistic spiderlings, with a four-fold extreme. Perhaps the low frequency of these cannibalistic broods is indicative of the usual low fitness (to the parents) of this condition. The increased size of the cannibals probably decreases the probability that they will balloon away from their nest site, thus increasing the probability of this trait concentrating within isolated populations. However, in contrast to this prediction, the phenomenon was uniformly rare but widespread in the present study.

Nest or egg-sac cannibalism could function as a radical alternative to ballooning under temporary and unpredictable conditions, in that it, too, provides a few spiderlings with an early supply of food. However, ballooning young in the study area face a particularly unfavorable probability of success, given the dominance of forest and water in the region.

The low frequency of cannibalism within the nests suggests that under most circumstances it does not yield strong advantages and may even be disadvantageous. Following Hamilton's (1964) argument for inclusive fitness, $-k < 1/r$, where k is the change in fitness of the victim divided by the gain in fitness of the cannibal, with r being the coefficient of relationship of the two individuals, Eickwort (1973)

noted that full sibs with equal initial fitnesses would present the most stringent conditions. Typically, female *Misumena* in these populations mate only once (Morse 2010), and their eggs and newly emerged young are of similar size (Morse 1993a), suggesting that they experience these stringent conditions. In young *Misumena* this advantage could result from reaching a larger size before overwintering, since larger individuals (later instars) appear to overwinter more successfully than smaller ones (Morse 2007). If females mated more than once, conditions would be less stringent. Second matings sometimes occur in the laboratory (Morse 2010), but probably seldom take place in the field in these populations, since densities are low and females aggressively attack males shortly after they first mate (Morse & Hu 2004). Thus, the low frequency of nest cannibalism observed matches the predictions from theory. Unfortunately, I do not know whether the cannibalistic broods resulted from polyandrous mothers.

Although this note involves only a small proportion of the many individuals analyzed, the overall sample size allows me to estimate the frequency of an uncommon trait in both space and time. Since cannibalism is reported from a wide range of taxa (Fox 1975; Polis 1981; Elgar & Crespi 1992) and is frequently compared between sibs and non-sibs, its presence in a species that often exhibits a short period of sociality subsequent to emergence from its natal site (D.H. Morse 2011) provides insight on a species intermediate between social or semi-social forms and forms that never congregate.

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SHORT COMMUNICATION

An unusually dense population of *Sphodros rufipes* (Mygalomorphae: Atypidae) at the edge of its range on Tuckernuck Island, Massachusetts

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Abstract. We counted and measured *Sphodros rufipes* (Latreille 1829) pursewebs in two survey plots on Tuckernuck Island, Massachusetts. Our objectives were to quantify web density, record physical web characteristics and determine the main components of *S. rufipes'* diet. We counted 479 webs in the two plots and report web densities between 0.058 and 0.18 webs/m^2, denser than previously reported populations. Webs were not distributed evenly, and densities ranged from 0 to 0.38 webs/m^2. Aggregation indices suggest that webs are aggregated on a landscape level, but are more evenly distributed at a local level. Contrary to most previously published literature on *S. rufipes,* we noted the predominance of the grass-like sedge, *Carex pensylvanica,* rather than trees, as a web support. Coleopterans and isopods made up 79 percent of the prey parts collected from 56 pursewebs.

Keywords: Purseweb spiders, web density, diet, spatial distribution

Most spiders in the genus *Sphodros* (family Atypidae) build vertical, tube-like webs that extend from below the soil surface to attach to the trunk of a tree or other solid surface (Gertsch and Platnick 1980). The aerial portion of the tube is usually well camouflaged by the spider with soil particles and debris. There are two *Sphodros* species in New England, USA. *Sphodros rufipes* (Latreille 1829) builds a vertical tube of silk and usually attaches it to the base of a deciduous tree (Hardy 2003). Males of this species have completely red legs, whereas females are all black. *Sphodros niger* (Hentz 1842) is a more cryptic species that usually constructs the 'aerial' portion of its web horizontally and, at least on Cape Cod, Massachusetts, underneath pine duff and leaf litter (Edwards & Edwards 1990). Males and females of this species are all black. *Sphodros rufipes* is a southern species reported in the literature as far north as Block Island, Rhode Island, while *S. niger* is a more northern species that occurs as far south as North Carolina and extends into Canada (Gertsch & Platnick 1980).

Most likely due to their cryptic lifestyle, previous researchers have only described attributes and behaviors that can be studied with small numbers of *Sphodros* spiders such as mating, prey capture, web placement, and web-building behaviors (e.g., McCook 1888; Muma & Muma 1945; Coyle & Shear 1981; Coyle 1983; Edwards & Edwards 1990; Hardy 2003). The only population-level study we are aware of was conducted over a two-year period in eastern Kansas on populations of *S. niger* and *S. rufipes*. Results were inconclusive, because the populations appeared to suddenly decline. To our knowledge, no other demographic data exist for *Sphodros* species.

Tuckernuck Island, 50 km south of Cape Cod, Massachusetts, consists of 3.3 km^2 of private property located 2.9 km west of the larger island of Nantucket and 14 km east of the even larger island of Martha's Vineyard. The largest mammal is white-tailed deer, and there are no large scavengers or predators, such as raccoons, skunks, or foxes.

In 2006, during an ongoing spider species survey of Tuckernuck Island that included five hours of ground searching, we confirmed the presence of *S. rufipes* in the form of numerous pursewebs in grassy areas of the island. *Sphodros rufipes* has been known locally for many years to occur on Tuckernuck (D. Brown pers. comm.). We excavated specimens (all female) in their webs to confirm species identity as *S. rufipes* rather than *S. niger* (Gertsch & Platnick 1980). During the summer of 2008 we returned to Tuckernuck with the objectives to estimate *S. rufipes* colony density, record web characteristics, and collect prey parts for diet analysis.

We made two trips to the island on 5–8 June and 17–20 August 2008. We counted webs in a 50 × 50 m plot on the western side of the island (southwest corner 41.304558°N, 70.26798°W) and a 37 × 50 m plot on the eastern side (smaller due to time constraints) (southwest corner 41.29922N, 70.24516°W). Each plot encompassed a previously identified aggregation of pursewebs. The western site was located on a western-facing hill covered in grasses and scattered heath shrubs. The eastern site was a flat area with a pitch pine stand (*Pinus rigida*) surrounded by extensive black huckleberry clones (*Gaylussacia baccata*) and patches of grassland. Neither site was near open water. The substrate at both sites was sandy loam. We assigned a coordinate system to each plot and began counting webs starting at the southwest corner designated as (0 m, 0 m) (Fig. 1). Walking up and down the north axis we counted webs within a 1m-wide path, starting a new path to the east. In this way, we zig-zagged through the plot parallel to the east axis. We held a 1m^2 quadrat frame to measure the meter-wide path as we walked, and we used survey flags to mark our previous path and line us up for the next pass through the plot. We recorded the location of each web by measuring its distance along the north and east axes.

In addition to the plots, we used a random searching protocol to assess how likely one is to find more than one web in a given area. After locating a web, we walked in three random directions, each for a random distance between zero and 50 m, and counted the number of webs we encountered. In all web encounters, we assumed that any web that was cylindrical rather than flattened was occupied.

Within and around the eastern and western plots we collected the remains of prey items from 56 webs for diet analysis. These prey remnants consisted of disarticulated sclerotized arthropod parts, usually hanging from silk threads at the top of a web.

Our results suggest that the *S. rufipes* population on Tuckernuck is very large. We counted a total of 479 webs, 146 in the west and 333 in the east (Fig. 1). Dividing by the surveyed area (2,500 m^2 in the west and 1,850 m^2 in the east) gives a density of 0.058 webs/m^2 in the west and 0.18 webs/m^2 in the east. We used APACK 2.23 to calculate aggregation indices for each site (Mladenoff & DeZonia 2004). This software provides both a class-specific aggregation index (AI) and a

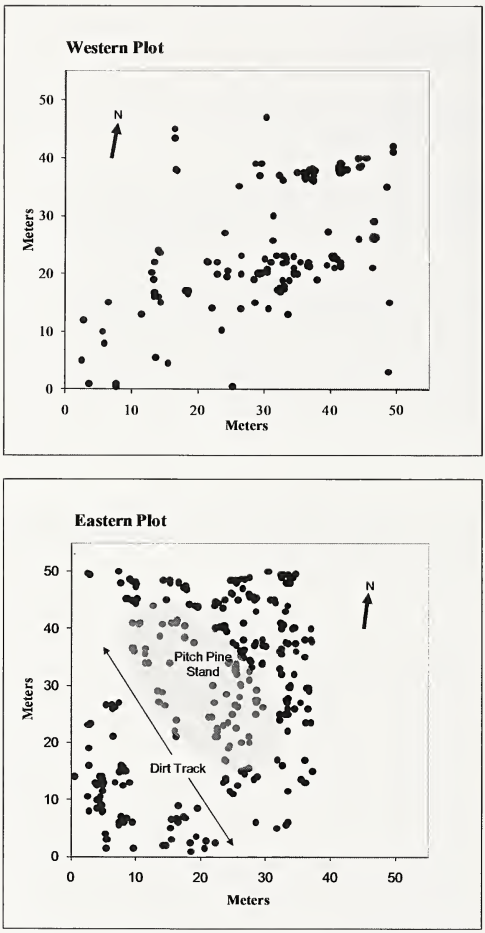

Figure 1.—Spatial distribution of *S. rufipes* webs (dots) plotted to within the nearest decimeter at the sampled western and eastern plots (upper and lower graphs, respectively).

landscape aggregation index (AI_L) with values between zero (disaggregated) and one (completely aggregated) (He et al. 2000). The AI_L represents the level of aggregation for both quadrats that contained webs and those that did not. At 1-m resolution, the web specific AI for the western site is 0.265 and the AI_L is 0.938. The web specific AI for the eastern site is 0.294 and the AI_L is 0.827, also at 1-m resolution. On a landscape level, the webs are fairly aggregated, but the web specific AIs suggest that webs are relatively dispersed.

To our knowledge, the Tuckernuck population occurs in colonies that are denser than other reported populations. For comparison, we compiled web numbers and, when available, the sampled area reported by other researchers. Poteat (1889) studied a population that containcd 0.04 webs/m^2 in North Carolina, while Hardy (2003, pers. comm.) studied one with a density of 0.01 webs/m^2 in Louisiana. Morrow (1986) in Kansas and Tom Chase (pers. comm.) on Martha's Vineyard, Massachusetts, studied populations that appeared to have more than one hundred webs in unmeasured areas. On Tuckernuck, the density at the eastern site (0.18) is more than four times Poteat's (1889) population density and 14 times the density Hardy (2003) describes. Our random search protocol showed that *S. rufipes* are not usually found alone or in isolated groups. We came across 12 additional webs outside the study plots, located in seven groups (each group contained between one and three webs within one m^2) spread across the island. We used our random searching protocol at these seven sites and located an additional 17 webs. We located additional webs at five of the seven groups (71%). Our success at finding more webs after locating one web or a small group of webs, suggests that *S. rufipes* on Tuckernuck occur in groups ranging from small aggregations to large colonies.

Vegetation used for web attachment is unusual on Tuckernuck. At the western plot, 83 percent of the webs were attached to non-woody objects (predominantly Pennsylvania sedge, *Carex pensylvanica*), and 16 percent were attached to a woody shrub. The average aerial web length with standard error was 11 ± 0.36 cm, but the distance from the ground to the top of any one web varied greatly (1–15 cm). In the eastern plot, 51% of the webs were attached to non-woody objects (again, predominantly *C. pensylvanica*), and 41% were attached to a woody shrub (predominantly *Gaylussacia baccata*). One of these webs was attached to a pitch pine (*Pinus rigida*) (25 cm diameter at breast height). This is unusual, for pines are not mentioned as web supports in any other study. Another 8% were attached to other objects such as a dead leaf, a dead log, or dead pine needles. The webs were on average 9.9 ± 0.88 cm long and the height from the ground to the top, again, varied greatly (0.5–15 cm).

There is only one previous report of *S. rufipes* using grass as a web support (Muma & Muma 1945), and most studies describe the spiders using trees. Hardy (2003) reported that *S. rufipes* in his study area used deciduous trees and avoided coniferous trees. Our findings strongly support a view that *S. rufipes* will use whatever support is available, even the rare conifer. Deciduous trees (mostly oaks) exist on Tuckernuck and form a centrally located forest, but in cursory surveys we did not find any webs attached to these trees. Large oaks were not present in our survey plots. Spiders did use the small woody shrub *Gaylussacia baccata*. Coyle & Shear (1981) noted that *S. rufipes* in Florida preferred smaller trees (< 10 cm) to larger ones.

We found prey remnants on 50% of webs (n = 111). Coleopterans and isopods were the most abundant prey items, found on 42% and 38% of the sampled webs, respectively (sampled webs refer to webs that contained prey parts). The most common coleopterans were Scarabaeidae (43% of coleopteran specimens) and Elateridae (17%). We found several other orders represented on only a few webs, including Diploda (1.8% of webs), Opiliones (3.6%), Araneae (7.1%), and Hymenoptera (14%). Our data are similar to those of Coyle & Shear (1981) and Muma & Muma (1945), who also collected prey parts from *S. rufipes* webs.

A possible explanation for the high densities we observed on Tuckernuck is low predation rates. We did not find evidence of any predation, and there are no mammal scavengers on the island. However, predation on *S. rufipes* webs has been observed on Block Island, R.I. in late March (E. Edwards, pers. comm.). Edwards found webs pulled up and dug out of the ground, probably by ring-necked pheasants. To our knowledge, there are no pheasants on Tuckernuck.

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SHORT COMMUNICATION

Does allometric growth explain the diminutive size of the fangs of *Scytodes* (Araneae: Scytodidae)?

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Abstract. Spitting spiders eject silk and glue from their fangs when attacking prey. The ejection is complete in less than 35 ms and involves high-frequency fang oscillations that can approach 1700 Hz. Because of Newtonian physical constraints, these oscillations, which cause the spit to be dispersed in a zigzag pattern, could not occur at such high frequencies if the fangs themselves were not very small. We hypothesized that allometric neoteny, in which the developmental rate of a structure is retarded relative to the changing overall size of the growing individual, could explain (in an ontological sense) the small fangs of adult spitting spiders. We measured the fangs, chelicerae, carapaces, and sterna of many sizes of spitting spiders, *Scytodes thoracica* (Latreille 1802a), brown recluse spiders, *Loxosceles reclusa* Fertsch & Mulaik 1940, and wolf spiders, *Varacosa avara* (Keyserling 1877), to discover whether the fangs of spitting spiders grow unusually slowly. Using sternum width as our proxy for spider size, we found that the carapaces of spitting spiders grow disproportionately fast but that the spiders' chelicerae and fangs grow at the same rate as their sterna. The growth patterns in *L. reclusa* and in *V. avara* differed both from each other and from *S. thoracica*. We evaluate these patterns and conclude that the diminutive fangs of adult spitting spiders do not constitute an instance of allometric neoteny.

Keywords: Spider predation, morphology, spitting dynamics, neoteny, ontogeny

Spitting spiders such as *Scytodes thoracica* (Latreille 1802a) (Araneae: Scytodidae) capture prey by entangling them in a mixture of silk and glue that the spiders eject through the venom duct in their fangs (Monterosso 1928; MacAlister 1960). The ejection is highly organized (Gilbert & Rayor 1985; Foelix 1996) and remarkably rapid. The ejected material, traveling at up to 28 m/s, forms an ordered zigzag pattern because the spider raises its chelicerae while its fangs oscillate, and an expectoration episode seldom lasts longer than 35 ms (Suter & Stratton 2009).

From a biomechanical perspective, the movement of the fangs is particularly interesting because their high frequency of oscillation (mean 826 Hz, maximum 1700 Hz) must be closely coupled to the mass of the fang, because it is the fang that must be accelerated at each extreme of its displacement. The rotational version of Newton's Second Law, tells us that

$$\tau = I \cdot \alpha \quad \text{or} \quad \alpha = \frac{\tau}{I} = \frac{\tau}{\Sigma mr^2}$$

angular acceleration (α) is the quotient of torque (τ) divided by the moment of inertia (I), where I is the sum of the products of mass and radius-squared (Σmr^2) for all particles making up the rotating structure. So, to achieve a given acceleration (and thus frequency of oscillation), as mass rises, torque must rise proportionately; or, for any given muscular or hydrodynamic torque, as mass rises, acceleration (and thus frequency of oscillation) must fall. (In the more familiar but less apt linear version of Newton's Second Law, $F = ma$, force is the equivalent of torque, acceleration replaces angular acceleration, and mass replaces the moment of inertia. In that version, like the rotational one, acceleration is directly proportional to force and inversely proportional to mass.) In this unavoidable physical context, a spitting spider with smaller fangs can achieve a higher oscillation frequency than an otherwise comparable spider with larger fangs, or can achieve the same oscillation frequency with less effort than would be expended by an otherwise comparable spider. It is not unexpected therefore to find that spitting spiders have very small fangs relative to the spiders' overall dimensions (Figs. 7–11 in Suter & Stratton 2005).

In the study reported here, we sought to test whether or not the adult spitting spider's diminutive fangs can be attributed to neoteny, the retention of juvenile traits in mature organisms. We approached this ontogenetic problem through allometry. As animals grow, the dimensions of their various parts increase, but seldom do so at the same rates. Entirely isometric growth implies that all parts grow comparably fast, so that a doubling in femur length would be accompanied by a doubling in tibia length and a doubling in the distance between the anterior median eyes. In fully isometric growth, a young animal would have exactly the same shape as an adult. Allometric growth implies that some parts grow faster than others, so that a doubling in femur length might be accompanied by a tripling of tibia length but no change at all in the distance between the eyes.

Allometric growth is usually detected by evaluating the allometric equation

$$y = bx^a \quad \text{or} \quad \log y = \log b + a \log x$$

in which y and x are the dimensions of two structures or other measurable properties (e.g., metabolic rate) and a is the allometric coefficient. In a regression of log y on log x, the slope is a and the intercept is log b; when $a < 1$, growth is negatively allometric, when $a = 1$, growth is isometric, and when $a > 1$, growth is positively allometric (Huxley 1932; Smith 1980; Harvey 1982).

We hypothesized that the relatively diminutive fangs of adult *S. thoracica* were the result of a negative allometry in which the fangs grew more slowly than other parts of the spider's anatomy throughout the life of the spider; this would result in adult spiders with disproportionately small fangs. To test this hypothesis, we measured fang length (tip to hinge), chelicera width (maximum), sternum width (maximum), and carapace width (maximum) in spiders that varied in size from hatchlings to adults. Carapace width is often used as a proxy for spider size (Hagstrum 1971), but we elected to use sternum width instead because the carapace of scytodids is abnormally large due to the hypertrophy of the venom glands (Foelix 1996; Ubick et al. 2005; and Fig. 6 in Suter & Stratton 2005) and so would, a priori, be an inappropriate proxy.

Because spider growth is strongly dependent on prey ingestion rate and only loosely attached to the passage of time (Homann 1949;

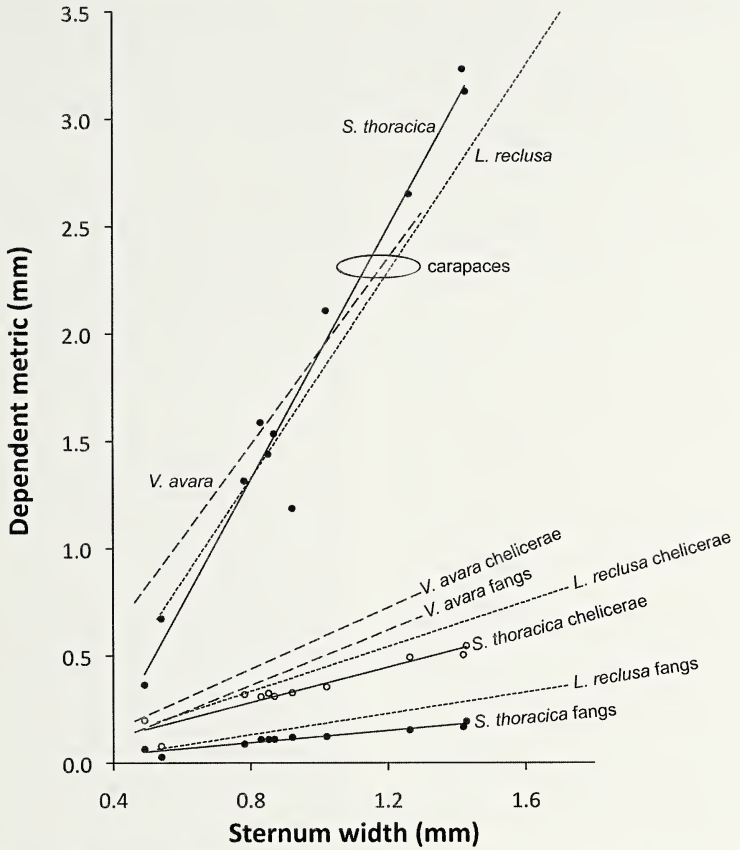


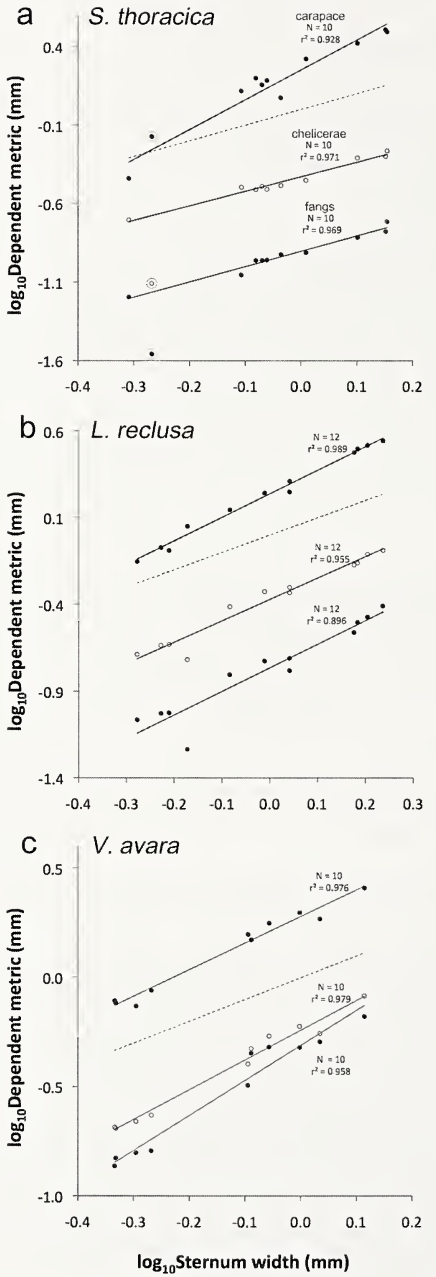
Figure 1.—Linear plots of the growth of fangs, chelicerae, and carapaces (relative to sterna) of *Scytodes thoracica* (solid circles and lines), *Loxosceles reclusa* (dotted lines), and *Varacosa avara* (dashed lines). To preserve visual clarity, data points are omitted for *L. reclusa* and *V. avara*. See Fig. 2 for the same data plotted as logarithms.

Higgins 1992, 2000; Sullivan & Morse 2004; Morse 2007), our independent variable throughout was sternum width rather than either time per se or developmental stage.

To facilitate measurement, we made calibrated images of whole spiders viewed under a dissecting microscope to get sternum and carapace dimensions, and we wet-mounted chelicerae and fangs on the stage of a compound microscope to make calibrated images of these two structures. We concentrated on three species: a spitting spider, *S. thoracica*, our focal species, collected in Oxford, Lafayette County, Mississippi; the brown recluse spider, *Loxosceles reclusa* Fertsch & Mulaik 1940 (Araneae: Sicariidae), another haplogyne species relatively closely related to the spitting spiders, collected from a variety of sites in Marshall and Lafayette Counties in Mississippi; and a wolf spider, *Varacosa avara* (Keyserling 1877) (Araneae: Lycosidae), a cursorial entelegyne spider distantly related to the spitting and recluse spiders, collected from Abbeville, Lafayette County, Mississippi.

Figure 1 shows the relationships between sternum width and the other dimensions we measured in the three species for which we collected developmental series. In each case, carapace width, chelicera width, and fang length increased approximately linearly with sternum width, our proxy for spider size. The relationships elucidated by applying the allometric equation, between the $\log_{10}$ of sternum width and the $\log_{10}$ of the other measures, varied interestingly among the three species we studied (Fig. 2, Table 1).

As expected from the spitting spider's hypertrophied venom glands and consequently enlarged cephalothorax (Foelix 1996; Suter & Stratton 2005; Ubick et al. 2005), the spitting spiders' carapaces grew with positive allometry (slope ± 95% CI = 1.90 ± 0.43, significantly greater than the isometric slope of 1.00). Their carapaces also grew more rapidly, in relative terms, than those of the brown recluse spiders (slope = 1.36 ± 0.10) and the wolf spiders (slope = 1.22 ± 0.16). In all three species, carapace growth was more rapid than sternum growth (slope > 1.00).



In the spitting spiders, fang and chelicera growth rates were indistinguishable from sternum growth (slope ~ 1) and were thus apparently isometric. In contrast, the fangs and chelicerae of the brown recluse spiders showed positively allometric growth rates (slopes > 1) that were not significantly different from the growth rate of the carapace. In *V. avara*, the wolf spider, the fangs and chelicerae grew with positive allometry (slopes > 1), with the fangs growing fastest.

Our hypothesis was that the fangs of adult *S. thoracica* are small because their growth was slow relative to the growth of other structures and thus relative to growth of the body as a whole. Rejecting this hypothesis would require both a) that the fangs of spitting spiders grow as fast or faster than the body as a whole and b) that we chose a suitable proxy for body size. The data (Fig. 2, Table 1) show that the fangs, chelicerae, and sternum of spitting spiders grow at the same rate (slope ~ 1), while carapace width grows markedly faster. Thus we may need to reject our hypothesis because we have satisfied one (a, above) of the necessary criteria for rejection. The data (Fig. 2, Table 1) also show that comparing the growth of other structures vs. the growth of the sternum can detect instances of non-isometric growth that are either expected (enlargement of the spitting spider's cephalothorax) or are consonant with our impressions from other studies (the large relative size of adult wolf spider's chelicerae and fangs; Rovner 1980; Walker & Rypstra 2001). This satisfies the other (b, above) of the necessary criteria for rejection.

We must, therefore, reject our initial hypothesis and accept the alternative that, although the fangs of *S. thoracica* grow slowly relative to the enlarged cephalothorax, the fangs do not grow more slowly than would be expected in isometric growth. Thus allometric neoteny, in which the developmental rate of a structure is slowed relative to the changing overall size of the growing organism (Gould 1977; McNamara 1986), cannot explain the small size of the spitting spider's fangs and we must search elsewhere for an explanation.

Because the fangs of hatchling and adult spitting spiders have the same relative size, the explanation of small fang size, even among the smallest spitting spiders, may be found in the family's phylogeny rather than in the ontogeny of the individual spiders.

Because details of that evolutionary path remain obscure, we cannot justify an assertion that the unusually small fangs of spitting spiders evolved in support of the fangs' function in ejecting spit while oscillating at high frequency. Among haplogynes, for example, the fangs of *Artema atlanta* Walckenaer 1837 (Pholcidae) are no larger relative to sternum width (unpublished data) than are the fangs of the spitting spider; because these two species are in the same clade within the Haplogynae, and the pholcids do not spit while the scytodids do, it is quite possible that small relative fang size evolved first in an ancestor shared by both species. If that is the case, then the ancestors of modern scytodids merely took advantage of the pre-existing condition while other components of spitting physiology and morphology were evolving.

←

Figure 2.—Logarithmic plots of the growth of fangs, chelicerae, and carapaces (relative to sterna) in three spiders. *S. thoracica* (a) showed significant positive allometry in the growth of its carapace, but its chelicerae and fangs grew at the same rate as the sternum. (Data indicated by large open circles are excluded from the linear fits because they are clear outliers: for the carapace and fang fits, r^2 improved from 0.75 and 0.79, respectively, to 0.97 for each when the outliers were excluded.) Growth rates in *L. reclusa* (b) were positively allometric relative to the sternum and the slopes of the lines for carapace, chelicerae, and fangs were not different from each other. Growth rates in *V. avara* (c) were also positively allometric, with significant slope differences among carapaces, chelicerae, and fangs. Dashed lines have slopes of 1.0. Slope analyses are shown in Table 1.

Table 1.—Slopes, slope comparisons, and 95% confidence intervals of the log-log relationships shown in Fig. 2.

Spider	Structure	Slope	95% CI
S. thoracica	Carapace	1.901	1.469–2.334
	Chelicera	0.926	0.796–1.056
	Fang	0.976	0.833 to 1.120
	$F_{2,24}$	21.388	
	P	< 0.0001	
L. reclusa	Carapace	1.359	1.259–1.460
	Chelicera	1.235	1.046–1.425
	Fang	1.361	1.035–1.688
	$F_{2,30}$	0.507	
	P	0.608	
V. avara	Carapace	1.221	1.064–1.378
	Chelicera	1.365	1.201–1.530
	Fang	1.607	1.333–1.881
	$F_{2,24}$	4.787	
	P	0.018	

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SHORT COMMUNICATION

Anelosimus oritoyacu, a cloud forest social spider with only slightly female-biased primary sex ratios

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Abstract. We examine the social characteristics and sex ratio of the recently described *Anelosimus oritoyacu* Agnarsson 2006. We find that this spider, whose nests occur on tree crowns and bushes in open fields near Baeza, Ecuador, lives in colonies that may contain from one to several thousand adult females and their progeny. It differs from most other social congeners in that it occurs at relatively high elevations (1800–1900 m) and its primary sex ratio, 2.5 females per male, is the least biased of any known social species in the genus. The low sex ratio bias may reflect a low colony turnover rather than high gene flow among colonies, as the colonies occurred in complexes that were few and far between, but appeared to be long-lived. The relatively small body size of adult females and a web that appears to allow the capture of insects from all directions, combined with individual and group foraging, may allow the formation of large colonies at an elevation where insects, albeit abundant, are for the most part small.

Keywords: Ecuador, Theridiidae, cooperation, life cycle, quasisocial, subsocial

The genus *Anelosimus* Simon 1891 is of particular interest in the study of spider sociality because it contains the largest number of non-territorial permanent-social (or quasisocial) species of any spider genus (Avilés 1997; Agnarsson 2006; Lubin & Bilde 2007). Among these, *Anelosimus eximius* Keyserling 1884, *Anelosimus domingo* Levi 1963, *Anelosimus dubiosus* Keyserling 1881, *Anelosimus guacamayos* Agnarsson 2006, *Anelosimus lorenzo* Levi 1979, and *Anelosimus rupununi* Levi 1956 have been the subject of one to several studies (e.g., Fowler & Levi 1979; Rypstra & Tirey 1989; Rypstra 1993; Avilés & Tufiño 1998; Marques et al. 1998; Avilés & Salazar 1999; Avilés et al. 2007; Purcell & Avilés 2007; Yip et al. 2007). Here we report on a new non-territorial permanent social *Anelosimus*, recently described by Agnarsson (2006) as *Anelosimus oritoyacu*. We show that although this species exhibits social organization similar to that of other social *Anelosimus* spiders, it presents some interesting differences. Along with *A. guacamayos*, *A. oritoyacu* occurs at what appears to be the elevational range limit for permanent sociality in this genus (Avilés et al. 2007) and its sex ratio is the least biased among known social *Anelosimus* (Avilés & Maddison 1991; Avilés et al. 2007). Here we present a brief account of the size and structure of *A. oritoyacu*'s nests and colonies, informal observations on the cooperative nature of its societies, and, given the relevance of sex ratios as indicators of population structure (Williams 1966; Nagelkerke & Sabelis 1996; Hardy 2002), estimates of its primary and tertiary sex ratios.

Location of nest complexes seen.—Over a period of six years (January 2002–June 2008) we located eight areas, all within a 10 km radius of Baeza, Ecuador (0°27′S, 77°53′W; 1800–1900 m elev.), that contained from 1–12 *A. oritoyacu* nests (median 3.5) each, for a total of 55 nest records (Table 1). When more than one nest was present within these areas, nests were typically clustered within meters of one another in what we refer to as "nest complexes." Distances between identified nest complexes ranged from 25 m to 3.5 km. Most nests were located on the crowns of trees or on bushes growing on open hillsides or roadsides. The nest complexes appeared remarkably stable over time—at two of the sites initially discovered in 2002, nests were still present in 2007 and 2008 (Table 1). In contrast, nest complexes in species such as *Anelosimus eximius* rarely last more than 2–3 years (L. Avilés unpublished data), and those of species such as *Theridion nigroannulatum* Keyserling 1884 usually last less than a year (Avilés et al. 2006)

Nest and web structure.—*A. oritoyacu*'s nests differed from those of most other social species in the genus in lacking a well differentiated basal basket and extensive superior prey capture webbing, as depicted, for instance, for *A. eximius* by Yip et al. (2008, fig. 1; see also photo in Avilés et al. 2001, fig. 9). Instead, *A. oritoyacu*'s webs consisted of a core area surrounding a piece of vegetation and prey capture strands running away from the core, including inferiorly from it (Fig. 1), much like the nests of *A. rupununi* (Avilés & Salazar 1999), a canopy species. Also as in *A. rupununi*, *A. oritoyacu*'s silk was of a lighter texture and whiter coloration than in most other congeneric species. This web structure may reflect the position of the webs on tree crowns, and the need to capture insects flying from the side and below the nests. *A. oritoyacu*'s nests, as well as those of *A. rupununi*, thus have characteristics that appear a response to the canopy location preferred by these species. The nests we observed ranged broadly in size. At least two nests, but possibly as many as seven, of the 55 recorded contained either a single adult female or what appeared to be the clutch of a single female. The majority of nests, however, were considerably larger. Several nests in the first nest complex seen in January 2002, for instance, measured on the order of 3–4 m in diameter and probably contained several thousand individuals. Among 20 nests measured (two in 2002, three in 2004, eight in 2007, and seven in 2008, from one, two, three, and five different colony complexes, respectively), the smallest measured 13 × 13 × 23 cm and the largest, 205 × 156 × 100 cm.

Colony age structure.—Of the five nests that we dissected (three in January 2002 from the initial nest complex found, one in December 2002 from a complex 300 m away from the former, and one in 2008 from a seven-nest complex found 500 m away from the original found), four contained a mix of juvenile and/or egg sacs, subadult, and adult spiders, suggesting that reproduction is not strongly synchronized within nests (Table 2); the remaining nest contained only subadult males and females (Table 2). We surveyed the age structure of seven additional nests, both in June–July (2004, 2008) and in December (2002, 2007) and found that adults and juveniles/egg sacs were present at both times. Taken together, these findings suggest that *A. oritoyacyu* either has a short generation time and/or that its life cycle is largely independent of the mildly seasonal rain patterns of the region (rainiest: May–July; least rainy: December–February; Neill 1999). Adult males were seen overlapping with adult females in at least eight colonies, suggesting that the opportunity for intracolony mating is present.

Table 1.—Location of nest complexes seen and the number of seen nests they contained at the date of inspection. Location code: BZ-TN = Baeza-Tena Road; BZ-LA = Baeza-Lago Agrio Road; BZ, TN and LA = towns of Baeza, Tena, and Lago Agrio, respectively. Km from Baeza shown after each location code.

Location code	Latitude	Longitude	Elevation (m)	Dates seen	# Nests in complex
BZ-TN 8.1	0.497083	77.873861	1822	Jan-02	4
				Dec-02	few
				Dec-07	several
				Jun-08	several
BZ-TN 8.1 +333 m	0.4955	77.876306	1881	Dec-02	2
BZ-TN 1.0	0.46322	77.87662	1866	Dec-02	12
				Dec-07	4
				Jun-08	3
BZ town				Jun-04	1
BZ-TN 4.5	0.4729	77.86819	1848	Dec-07	1
				Jun-08	2
BZ-LA 2.4	0.45157	77.88392	1818	Jul-04	2
BZ-LA 2.6	0.451395	77.88954	1823	Jun-08	1
BZ-LA 3.0	0.45152	77.88399	1842	Dec-07	8
				Jun-08	7
BZ-LA 3.0 + 25 m	0.45152	77.88399	1842	Dec-07	4
				Jun-08	4

Clutch size and sex ratio.—In 2004, we collected egg sacs from a single large colony and used the method described by Avilés & Maddison (1991) to sex the embryos they contained. Egg sacs were off-white in color, averaged 3.9 ± 1.2 mm in diameter (n = 5), and contained between 16 and 46 eggs (n = 11, median = 37, mean = 34, SE = 3.13). In cytological spreads of individual embryos we counted the number of chromosomes contained in at least three dividing cells to determine whether the individual was male or female (n = 130, four egg sacs, Table 2). As in other species in the genus, males had 22 and females, 24 chromosomes (20 autosomes plus two sex chromosomes for males, and four sex chromosomes for females). Samples with fewer than three scorable dividing cells were not included in this analysis. We found the primary sex ratio to be about 2.5 females to a male (Table 3). This sex ratio differs significantly from the expected 1:1 sex ratio of subsocial species (Avilés & Maddison 1991; but see Gunnarsson & Andersson 1992 for a solitary species with biased sex ratios) and from the 10:1 sex ratio found in other social *Anelosimus* species (e.g., *A. eximius* and *A. domingo*, Avilés & Maddison 1991; *A. guacamayos*, Avilés et al. 2007). The tertiary sex ratio of adult and subadult spiders from nests collected in 2002 and 2008 similarly showed a bias of between two and five females to one male (Table 1).

Spider size and instars.—We measured the length of the tibia + patella on leg pair 1 (TP1) and leg pair 2 (TP2), as well as the sternum length (SL) and weight for a haphazard sample of subadult and adult spiders collected from one nest belonging to the original complex seen in 2002. Lengths were measured to the nearest 0.1 mm using an SZH Olympus dissecting stereomicroscope. We measured weights to the nearest 0.0001g using a Mettler Toledo standard level balance. The average ± SE of each measurement is presented for each instar (Table 4). We found that *A. oritoyacu* males were adult at a size

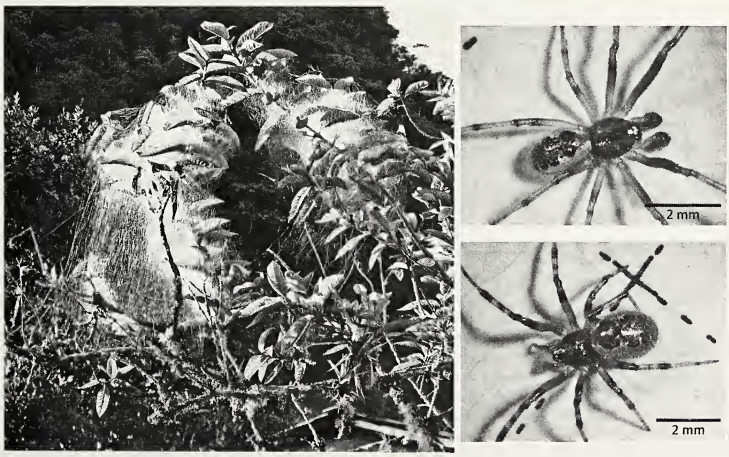

Figure 1.—*Anelosimus oritoyacu*'s nests photographed near Baeza, Ecuador, and photographs of adult male (above) and female (bottom) spiders. Note the different scales of the male and female photographs.

Table 2.—Colony age structure breakdown and the tertiary sex ratio (total number of adult plus subadult females / total number of adult plus subadult males) for five dissected *A. oritoyacu* colonies.

Colony	Collection date	Nest size (cm)	% Collected	Contents (number)							Tertiary sex ratio
				Ad m	Sub m	Ad f	Sub2 f	Sub1 f	Juvs	Sacs	
BZ-TN 8.1-1	6 Jan 2002	45 × 25 × 14	100	27	14	12	47	29	36	0	2.15:1
BZ-TN 8.1-2	6 Jan 2002	40 × 18 × 17	100	9	21	16	28	17	51	3	2.03:1
BZ-TN 8.1-4	6 Jan 2002	–	100	32	6	42	28	67	0	4	3.61:1
BZ-TN 8.1 + 333-1	17 Dec 2002	–	100	0	13	0	27		0	0	2.08:1
BZ-LA 3.0-7	20 Jun 2008	–	30	15	11	16	44	76	45	4	5.23:1

Table 3.—Primary sex ratio of *Anelosimus oritoyacu*, reported as the proportion of males among developing embryos in four egg sacs. The proportions are compared with 1:1 and 10:1 sex ratio expectations (right two columns) using either the binomial exact test for each egg sac (rows 1–4) and the total sample (row 5) or the weighted Z-transform method (last row), which combines the probabilities of the four egg sacs, with each sac weighted by the number of embryos scored to give more weight to more precise estimates, as recommended by Whitlock (2005).

Egg sac	Total embryos	Total scored	# of Males	Proportion of males	$P_{1:1}$	$P_{10:1}$
1	24	21	7	0.33	0.09	0.003
2	41	34	8	0.24	0.001	0.02
3	37	31	8	0.26	0.005	0.01
4	46	44	14	0.32	0.007	< 0.001
Total:	–	130	37	0.28	<< 0.001	<< 0.001
Mean:	–	32.5	9.25	—	Z_s	Z_s
St. Dev.:	–	9.47	3.2	—	0.04	0.01

Table 4.—Instar measurements for subadult and adult males and females. The mean is shown with the standard error in parentheses. Measurements include tibia + patella for leg pair 1 (TP1) and leg pair 2 (TP2), sternum length (SL) and weight.

Instar	*n*	TP1 (mm)	TP2 (mm)	SL (mm)	Weight (mg)
Male					
Subadult	6	1.25 (0.0224)	1.02 (0.0307)	0.7 (0.000)	3.53 (0.243)
Adult	7	1.86 (0.023)	1.39 (0.0254)	0.779 (0.0149)	3.87 (0.167)
Female					
First Subadult	4	1.43 (0.025)	1.16 (0.0239)	0.738 (0.0125)	3.43 (0.330)
Second Subadult	4	1.69 (0.375)	1.36 (0.0239)	0.9 (0.000)	4.43 (0.325)
Adult	14	2.129 (0.0266)	1.66 (0.0195)	1.04 (0.0116)	5.52 (0.229)

corresponding to the second subadult female instar (Table 3, Fig. 1), suggesting that males mature one instar earlier than females, as is the case with other tropical *Anelosimus* (e.g., Avilés 1986; Avilés et al. 2007).

Interestingly, *A. oritoyacu* appears to exhibit significantly less sexual size dimorphism than other Ecuadorian social *Anelosimus* (Fig. 2) (mean male: female body length ratio = 0.79 for *A. oritoyacu*; 0.68 for *A. guacamayos*; 0.66 for *A. domingo*; 0.65 for *A. eximius*; total body lengths of 15 to 31 specimens per species measured to the nearest 0.1 mm). This is due to adult *A. oritoyacu* females being relatively small compared to females in these other species (mean ± SE, *oritoyacu*: 3.65 ± 0.11 mm, $n = 7$; *eximius*: 4.84 ± 0.06 mm, $n = 21$; *guacamayos*: 4.04 ± 0.07 mm, $n = 21$; *domingo*: 3.49 ± 0.08 mm, $n = 15$), while *A. oritoyacu* males are relatively large (*oritoyacu*: 2.90 ± 0.19 mm, $n = 8$; *eximius*: 3.14 ± 0.08 mm, $n = 10$; *guacamayos*: 2.76 ± 0.12 mm, $n = 4$; *domingo*: 2.29 ± 0.08 mm, $n = 12$). The significance of this pattern is unclear.

Conclusions and discussion.—In conclusion, the size, duration, and demographic composition of *A. oritoyacu* colonies, including their female biased sex ratios, are consistent with this being a non-territorial permanent social species with colonies that last for multiple generations. The estimated 2.5 females per male primary sex ratio further suggests that some degree of intracolony mating must be taking place in this species, as is typical of species with this level of sociality (Avilés 1986, 1993, 1997). It is interesting, however, that *A. oritoyacu*'s sex ratio is the least biased among known permanent social *Anelosimus*, as other species typically exhibit sex ratios of 10:1 (*A. eximius*, *A. domingo*: primary sex ratio), 5:1 (*A. guacamayos*: primary sex ratio), and 3:1 (*A. dubiosus*: sex ratio among subadults to adults). Avilés (1993) showed through computer simulations that the most highly biased sex ratios arise when the degree of isolation of the colony lineages and their rate of turnover (i.e., rate of colony extinction and replacement) are the greatest. Sex ratios that are only slightly biased would thus arise if there were some degree of gene flow among the colonies' lineages and/or their rate of turnover were relatively low. Without genetic data to assess population structure on *A. oritoyacu*, at the moment we cannot ascertain which of these two (or combination of these two) factors plays the most important role in determining the low sex ratio bias of this species. However, the fact that *A. oritoyacu*'s nest complexes were few and far between does suggest that the likelihood that dispersing males would find nests of unrelated females (i.e., belonging to a different complex) are low to non-existent. On the other hand, the fact that *A. oritoyacu*'s nests and colonies appear relatively long-lived compared to those of other social *Anelosimus* suggests that a low rate of colony turnover may be the parameter most likely responsible for the low sex ratio bias observed, a prediction that requires further testing.

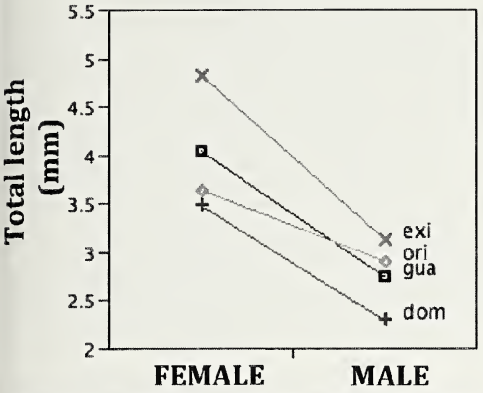

Figure 2.—Least square means for the total length of male and female spiders of four social *Anelosimus* species found in Ecuador (ex = *A. eximius*; ori = *A. oritoyacu*; gua = *A. guacamayos*; dom = *A. domingo*). Note that the size difference between *A. oritoyacu* males and females is significantly smaller than that found in the other three species, as confirmed by a significant interaction between species and sex ($F_{3,72}$ = I3.3; $P = < 0.0001$) in a mixed model ANOVA including, in addition to the two factors and their interaction, colony identity as a random effect.

Another interesting aspect of the biology of this species is that, along with *A. guacamayos* (which occurs at up to 1,940 m elev.), it occurs at the elevational range limit for sociality in the genus (Avilés et al. 2007). Our earlier studies (Guevara & Avilés 2007; Powers and Avilés 2007) suggest that absence of an abundant supply of large insects at high elevations and latitudes may restrict social *Anelosimus* species to low-to mid-elevation tropical moist forests. The reason is that large insects, which are caught cooperatively by larger colonies, are needed to compensate for a decline in the surface area per unit volume of the prey capture snares—and thus of the number of insect prey per capita—as colony size increases (Yip et al. 2007). So, how can *A. oritoyacu* manage colonies containing thousands of individuals at an elevation where there are proportionally few large insects compared to lower elevation areas where social *Anelosimus* thrive? We suggest at least three non-mutually exclusive hypotheses to be tested in future studies. 1) Because *A. oritoyacu* females are small compared to most other *Anelosimus* species (see above and Fig. 2), the supply of insects larger than the spiders may still be significant at the elevations at which it lives. 2) There may be proportionally less loss of surface area per unit volume of *A. oritoyacu*'s webs as colonies grow because its webs appear to capture insects from all directions, rather than just from above, as in the more typical *Anelosimus* species with a basal basket-shaped nest (e.g., *A. eximius*, see drawing in Yip et al. 2007). 3) Although insects are on average smaller at higher elevation cloud forest areas, such as the one we studied (e.g., Guevara and Avilés 2007), our earlier studies show that insect density (number of insects per unit area) in these areas is greater than in the lowland tropical rainforest (Powers & Avilés 2007), so that the overall biomass of potential prey is either the same (E. Yip & L. Avilés unpublished data) or somewhat greater (Powers & Avilés 2007) than at lower elevations. Given an abundance of small insects, through individual and cooperative prey capture, both of which we have witnessed (L. Avilés unpublished data), the spiders may be able to sustain large social colonies if other aspects of their fitness are substantially enhanced by group living. During the course of this study we obtained preliminary evidence that females may care indiscriminately for each other's egg sacs, as we witnessed multiple instances of egg sac switching over a 24-h period in artificially established groups (four) of five color-coded females and their sacs (L. Avilés unpublished data). Above and beyond any benefits that may arise from cooperative prey capture, offspring fitness could thus be enhanced by the availability of surrogate caregivers in the event of the mother's death (e.g., Jones et al. 2007). These are all ideas that will need to be formally explored in future studies.

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SHORT COMMUNICATION

Observations on hunting behavior of juvenile *Chanbria* (Solifugae: Eremobatidae)

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Abstract. Juvenile solifuges have rarely been observed hunting in natural conditions. We recorded the hunting behavior of juvenile third or fourth instar solifuges of the genus *Chanbria* (Eremobatidae) near lanterns set up in the Imperial Sand Dunes, Imperial County, California. At least 10 juveniles were observed between 22:50 and 01:40 h on 18–19 June 2010. The behavior consisted of nearly constant movement, abrupt stops or retreats, and quick excavation of the sand. The juveniles probed the sand using their pedipalps. One juvenile was observed to dig up an immature Hemiptera from just beneath the surface amidst the sand grains. Direct contact with other solifuges or arthropods occasionally triggered an immediate flight response.

Keywords: Solifugids, camel spiders, predation

The order Solifugae remains poorly studied (Punzo 1998a; Harvey 2003). This is largely due to difficulties in observing individuals in the wild, lack of success raising solifuges in captivity, and a generally low yield of specimens from field collection efforts (Punzo 1998a). Little is known about the behavior of early instars since few researchers have been successful raising solifuges to maturity in captivity, and even fewer studies document the behavior of juveniles in the wild (Punzo 1998a, 1998b). Herein we report observations on the hunting behavior of juveniles in the genus *Chanbria* (Solifugae: Eremobatidae). *Chanbria* currently includes *C. rectus* Muma 1962, *C. regalis* Muma 1951, *C. serpentinus* Muma 1951, and *C. tehachapianus* Muma 1962; all of which are psammophilic species found in southwestern United States and northwestern Mexico. This is the first record of hunting behavior for juvenile *Chanbria* and one of the very few records of hunting behavior in juvenile Solifugae. Muma (1966a), Wharton (1987) and Hrušková-Martišová et al (2007 (2008)) have previously reported observations on juvenile solifuges in natural conditions.

The observations occurred on 18–19 June 2010 in the Imperial Sand Dunes Recreation Area, Imperial County, California (32.94586°N, 115.14703°W). Since solifuges are known to be attracted to light (Cloudsley-Thompson 1977; Punzo 1998a), we set up three Coleman lanterns in a triangle on top of a sandy ridge. Each lantern was suspended on a wooden tripod to elevate it slightly above the ground. The lights were set up just at dusk (20:10 h). The sand ridge was situated between an open, unvegetated dune habitat and a sparsely vegetated desert habitat with small clumps of shrubs. Penultimate and juvenile solifuges approached the lights exclusively from the direction of the vegetated habitat and were first observed at 22:55 h. From that time until 01:40 h when observations ended, we observed at least 10 juveniles hunting under the pool of light.

Three of the juveniles were captured, preserved in 100% ETOH, and deposited in the arachnology collection of the Denver Museum of Nature & Science (#ZA.23696). These early instar juveniles were 4 mm from the anterior edge of the propeltidium to the posterior of the abdomen. The juveniles collected had three sets of malleoli. Since the first four nymphal stages of Eremobatidae exhibit three pairs of malleoli and do not develop the full complement of five pairs until the fifth instar (Muma 1966b), the juveniles observed in the field were no older than 4th instar nymphs. The loss of aggregative behavior only after the second instar molt (Cloudsley-Thompson 1977) suggests that the juveniles we observed in the field were third or fourth instars.

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The early instar juveniles moved in an apparently erratic search pattern. Their search was often interrupted by a quick, short retreat along their previous path, immediately followed by a vigorous excavation of the sand with their 2nd and perhaps also 1st pair of legs and chelicerae, creating a shallow bowl under the crust of the sand. The period of digging was variable. Some individuals dug for only a few seconds, while others paused, probed the hole with their pedipalps, and then immediately began digging again for a variable number of times until they began their search for another patch of sand to excavate. No visible sign on the surface of the sand gave us hints as to why the solifuges would pick a spot to dig. However, one specimen was seen to excavate a hemipteran nymph from just under the surface of the sand, and another was seen eating an aphid, though its excavation was not observed.

The pool of light attracted many different desert arthropods. When a young *Chanbria* directly contacted another arthropod of similar size, it typically showed avoidance behavior. Individuals appeared to run backwards, as has been reported for pseudoscorpions (Weygoldt 1969; de Andrade & Gnaspini 2003), although whether solifuges are capable of backward movement remains to be tested. This movement away from disturbance was sometimes followed by a very brief pause and a resumption of foraging. One of us (PEC) observed one juvenile standing still, vibrating its raised pedipalps. We do not know whether this behavior was a response to disturbance or a method for detecting airborne chemical cues.

Our observations suggest that juvenile *Chanbria* may use a combination of tactile and chemical cues to locate prey that are buried just beneath the surface of the sand. We suspect they may use chemosensory signals since we saw them reverse directions on several occasions and begin digging in areas they had just passed. Brownell & Farley (1974) showed that the malleoli function as chemoreceptors; thus, the juveniles were returning to areas that they had, presumably, just contacted with the malleoli. However, it is likely they also use tactile cues for prey localization; our observations of juvenile *Chanbria* support the use of pedipalps for tactile detection of prey. Substrate tactile cues have been shown to be involved in prey localization in other species of Solifugae (Muma 1966a; Wharton 1987).

Hrušková-Martišová et al. (2007 (2008)) reported on *Galeodes caspius subfuscus* (Birula 1890) and had unique observations of juvenile hunting behavior. Juveniles were observed to hunt exclusively on bushes, hanging on branches with their pedipalps extended forward. They were observed to catch flying prey, including Trichoptera. One of our observations in the field was a juvenile sitting still with pedipalps extended, vibrating, which may reflect a prey localization behavior similar to that seen in *G. caspius subfuscus*.

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SHORT COMMUNICATION

A new troglobitic *Eukoenenia* (Palpigradi: Eukoeneniidae) from Brazil

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Abstract. A new Brazilian species of the genus *Eukoenenia* is described from a single male specimen collected within the Archimedes Passini cave, a marble cave located in the municipal district of Vargem Alta, Espírito Santo. *Eukoenenia spelunca*, sp. nov., has six blades on the prosomal lateral organs and a unique shape of the genital lobes. Some morphometric parameters demonstrate the specialization of this new species to the cave environment.

Keywords: Neotropics, taxonomy, caves, troglomorphic

Palpigradi is one of the least known of the arachnid orders, and its phylogenetic position is problematic (Pepato et al. 2010). Historically, various authors (Hansen & Sörensen 1897; Petrunkevitch 1955; Weygoldt and Paulus 1979; van der Hammen 1982) have proposed different relationships with other groups of arachnids, but there is no consensus.

Within the Palpigradi, the most distinctive troglomorphisms are found in species of the genus *Eukoenenia* Börner 1901, which is also the most diverse and widely distributed genus. Representatives of the genera *Allokoenenia* Silvestri 1913, *Koeneniodes* Silvestri 1913, and *Prokoenenia* Börner 1901 sometimes have been found in caves, but in none of the cases have the species expressed adaptations related to the subterranean environment (Condé 1996).

Despite being one of the smallest arachnid orders (Harvey 2007), new palpigrade species are being regularly discovered and described (e.g., Moreno 2006; Barranco & Harvey 2008; Christian 2009). In recent years researchers have uncovered a variety of Palpigradi in several Brazilian caves (Souza & Ferreira 2010). Most of these species are new, and many are currently under study to determine their affinities. In the present work, a new Brazilian species of the genus *Eukoenenia* with troglomorphic traits is described from an adult male found walking on a speleothem in a marble cave in the municipal district of Vargem Alta, Espírito Santo.

METHODS

The specimen was examined by clearing it in Nesbit's solution and mounting it in Hoyer's medium on 3 × 1-inch glass slides using standard procedures developed for mites (Krantz & Walter 2009). All measurements are presented in micrometers (μm) and were taken using an ocular micrometer with a phase contrast microscope. Body length was measured from the apex of the propeltidium to the posterior margin of the opisthosoma. The areoles in some drawings represent the insertions of setae.

The following abbreviations were utilized, based on Barranco & Mayoral (2007): L, total body length (without flagellum); B, dorsal shield length; P, pedipalpus; I and IV, legs I and IV; ti, tibia; bta1, basitarsus 1; bta2, basitarsus 2; bta3, basitarsus 3; bta4, basitarsus 4; ta1, tarsus 1; ta2, tarsus 2; ta3, tarsus 3; a, width of basitarsus IV at level of seta r; er, distance between base of basitarsus IV and insertion of seta r; grt, tergal seta length; gla, lateral seta length; r, stiff seta length; t/r, ratio between length of basitarsus IV and stiff seta length; t/er, ratio between basitarsus IV length and distance to insertion of stiff seta; gla/grt, ratio between lengths of lateral and tergal setae; B/bta, ratio between lengths of prosomal shield and basitarsus IV; bta/ti, ratio between lengths of basitarsus IV and tibia IV. Setal nomenclature follows that of Condé (1974a, 1974b, 1981, 1984, 1988, 1989, 1992, 1993, 1994).

The specimen is lodged in the Coleção de Invertebrados Subterrâneos de Lavras, Departamento de Biologia, Universidade Federal de Lavras, Lavras, Minais Gerais (ISLA).

TAXONOMY

Family Eukoeneniidae Petrunkevitch 1955

Genus *Eukoenenia* Börner 1901

Koenenia Grassi & Calandruccio 1885:165 [junior primary homonym of *Koenenia* Beushausen 1884 (Mollusca: Bivalvia)].

Koenenia (*Eukoenenia*) Börner 1901:551.

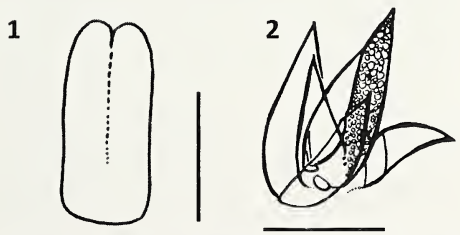
Type species.—*Koenenia mirabilis* Grassi & Calandruccio 1885, by monotypy.

Eukoenenia spelunca new species

(Figs. 1–15)

Material examined.—Brazil: *Espírito Santo*: Holotype adult male, Archimides Passini cave (collected from a speleothem), Vargem Alta (UTM 285168,01; 7711062,66), 15 September 2005, R.L. Ferreira (ISLA 850).

Diagnosis.—*Eukoenenia spelunca* differs from all other species of the genus by the following combination of characters: prosomal lateral organs with 6 blades; six setae on the basitarsus IV with a single proximal sternal seta; opisthosomal sternites IV–VI with 2 + 2 thickened setae (a_1, a_2) in middle of the opisthosoma between both normal slender setae (s); and male genitalia with 11 + 11 setae on first



Figures 1, 2.—*Eukoenenia spelunca* new species, holotype male: 1. Frontal organ, dorsal view; 2. Lateral organ, dorsal view. Scale bars 20 μm (Fig. 1), 20 μm (Fig. 2).

Figures 3–5.—*Eukoenenia spelunca* new species, holotype male: 3. Propeltidial chaetotaxy; 4. Metapeltidial setae; 5. Deuto-tritosternal setae. Scale bars 100 μm (Fig. 3), 40 μm (Fig. 4), 20 μm (Fig. 5).

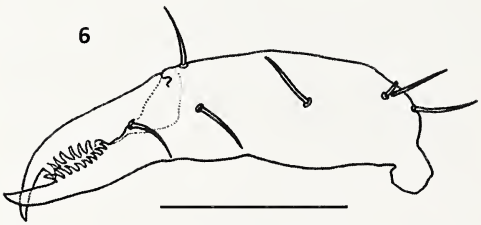
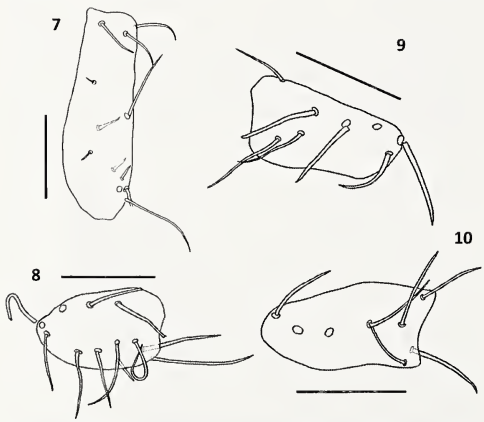
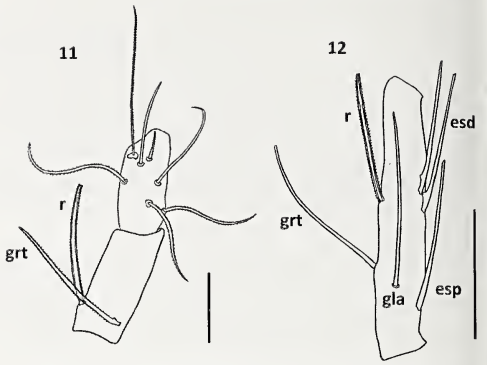


Figure 6.—*Eukoenenia spelunca* new species, holotype male: 6. Chelicerae. Scale bar 100 μm.



Figures 7–10.—*Eukoenenia spelunca* new species, holotype male: 7. Coxa I; 8. Coxa II; 9. Coxa III; 10. Coxa IV. Scale bars 60 μm.



Figures 11, 12.—*Eukoenenia spelunca* new species, holotype male: 11. Basitarsus 3–4 of leg I; 12. Basitarsus IV. Scale bars 40 μm (Fig. 11), 60 μm (Fig. 12).

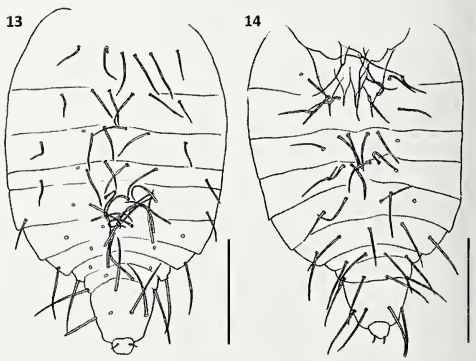
lobe (and 2 + 2 sternal setae), 4 + 4 setae on second lobe, and 4 + 4 setae on third lobe.

Description.—*Prosoma:* frontal organ with two branches, blunt apically and each 4.4 times longer than wide (27.5 μm/6.25 μm) (Fig. 1). Lateral organ with 6 pointed parallel blades, each 6.5 times longer than wide (32.5 μm/5 μm) (Fig. 2).

Propeltidium with 10 + 10 setae (Fig. 3). Metapeltidium with 3 + 3 setae (t_1, t_2, t_3) each of different length, inner seta shortest (65 μm, 75 μm, and 67.5 μm) (Fig. 4). Deutotritosternum with 5 setae in U-shaped arrangement (Fig. 5).

Chelicerae: with 9 teeth on each finger; 4 dorsal setae, 1 lateral seta, and 1 seta inserted near the row of teeth of the second segment (Fig. 6).

Legs: chaetotaxy of coxae I–IV: 11, 8, 12 and 8 (Figs. 7–10). Basitarsus 3 of leg I 2.3 times longer than wide, with 2 setae (*grt* 67.5 μm; *r* 77.5 μm). Seta *r* longer than segment (65 μm /77.5 μm, t/r = 0.8), inserted in proximal half and surpassing hind edge (27.5 μm/60 μm, s/er = 0.45) (Fig. 11). Basitarsus of leg IV 5.6 times longer than wide, with 6 setae (2 *esd*, *esp*, *gla*, *grt* and *r*), *btalti* 0.91. Stiff seta



Figures 13, 14.—*Eukoenenia spelunca* new species, holotype male: 13. Opisthosoma, dorsal view; 14. Opisthosoma, ventral view. Scale bar 150 μm.

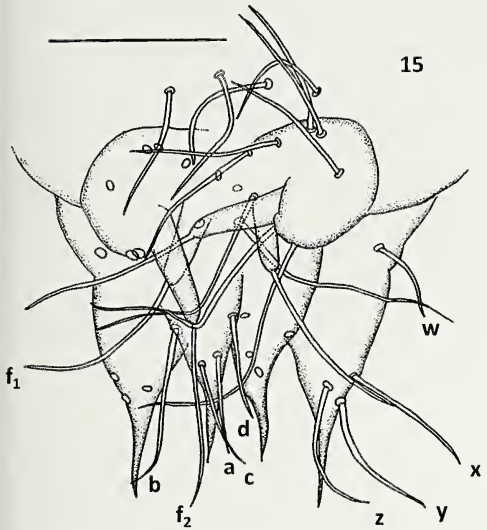


Figure 15.—*Eukoenenia spelunca* new species, holotype male: 15. Male genitalia. Scale bar 60 µm.

r 2.2 times shorter than tergal edge of article (127.5 µm/57.5 µm, *tlr* = 2.2) and inserted in its distal half (127.5 µm /72.5 µm, *tler* = 1.75). Seta *esp* proximally inserted, followed by *gla* and *grt*, more or less at the same level, all of them in proximal half (Fig. 12).

Opisthosoma: tergites II–VI with 3 + 3 setae each, 2 pairs of setae (t_1, t_3) between both slender setae (*s*). Tergites VII–VIII each with 2 + 2 setae (Fig. 13). Sternite III with 2 + 2 setae. Sternite IV–VI each with 2 + 2 thickened setae (a_1, a_2) in middle of the opisthosoma between both normal slender setae (*s*). Sternites VII–VIII with 2 + 2 setae and 2 + 1 + 2 setae respectively. Segments IX–XI each with 8 setae (Fig. 14).

Genitalia: with 2 + 2 external setae (st_1 and st_2) and 38 setae distributed in 3 lobes that form the genitalia of the male. First lobe with a rounded aspect, not being possible to identify a clear separation in the central region; with 11 + 11 setae (including 2 + 2 fusules in the distal margin); f_1 = 80–85 µm; f_2 = 100–95 µm. Second lobe subtriangular, with a simple and sharp apex (without bifurcation), with 4 + 4 setae (*a, b, c, d*). Third lobe also in a subtriangular form, well developed, with 4 + 4 setae (w, *x, y, z*), with a large, sharp and simple acute apical region (Fig. 15).

Dimensions (µm)*:* See Table 1.

Etymology.—Name given in apposition as a reference to the Corsican word *spelunca* meaning "cave."

Habitat.—Archimedes Passini cave is formed within marble and is located in the municipal district of Vargem Alta (Espírito Santo). This cave possesses approximately 150 m of linear development. Its topography is irregular and the more interior portion of the cave harbors a small drainage. The only individual of *E. spelunca* collected was walking on a stalagmitic floor, about 40 m from the only cave entrance. This area is isolated from the surrounding epigean environment, comprising a conduit with a low ceiling (about 1 m high) and a more stable microclimate. The surface of the stalagmitic floor where the palpigrade was collected was quite humid. The cave is located in the domain of the Brazilian Atlantic forest, but the area has been quite altered by anthropogenic activities, deforestation being very frequent in the area.

Table 1.—Measurements (µm) of selected body parts of the two type specimens of *Eukoenenia spelunca*.

Body part	Holotype
L	720
B	245
Pti	115
PbtaI	52.5
Pbta2	62.5
Pta1	32.5
Pta2	40
Pta3	50
Iti	117.5
Ibta1+2	100
Ibta3	65
Ibta4	62.5
Ita1	15
Ita2	32.5
Ita3	120
IVti	140
IVbta	127.5
IVta1	50
IVta2	57.5
A	22.5
Er	72.5
Grt	75
Gla	77.5
R	575
tlr	2.21
tler	1.75
gla/grt	1.03
B/btaIV	1.92
btaIV/ti	0.91

DISCUSSION

Among the species of Palpigradi found in South America, *Eukoenenia improvisa* Condé 1979 from French Guiana (Condé 1979a) has characteristics most in common with *E. spelunca*. Such characteristics include the presence of 6 setae on the basitarsus of leg IV (presence of only a seta *esp*), the chaetotaxy of the opisthosomal sternites IV–VI (2 + 2 thickened setae between both slender setae) and of the opisthosomal tergites II–VI (3 + 3 setae, two pairs of seta t between both seta *s*), presence of 5 setae in the deutotritosternum, and seta r inserted in the distal half of the basitarsus IV. However, some characteristics distinguish *E. improvisa* from *E. spelunca* such as the lateral organs formed by 4 elements, the disposition of the setae of the deutotritosternum, and the body dimension values. Although *E. improvisa* has a larger body size, *E. spelunca* has longer segments that form the pedipalp and legs I and IV, the former characteristic of edaphomorphic species and the latter with troglobitic species. Unfortunately, the characteristics of the genitalia cannot be compared, since the male of *E. improvisa* is not known (Condé 1979a). Despite these similarities with *E. improvisa*, a better knowledge of the intertropical species is necessary, based on males and females, so that it is possible to group them or to phylogenetically associate them.

The chaetotaxy of the opisthosomal sternites IV–VI of *E. spelunca* is also similar to that of *E. thais* Condé 1988 and *E. lyrifer* Condé 1992 (Condé 1988, 1992). In addition, the occurrence of 6 setae of the IV bta due to the presence of only one seta esp is also observed in *E. pauli* Condé 1979 (Condé 1979c).

The presence of 6 elements forming the lateral organs in *E. spelunca* is shared with other species found in caves such as *E. spelaea* (Peyerimhoff 1902) (5–6), *E. remyi* Condé 1974 (4–6), *E. maroccana* (6) and *E. maquinensis* Souza & Ferreira 2010 (6) (Peyerimhoff 1902; Condé 1974; Souza & Ferreira 2010).

The male genitalia of *E. spelunca* has 38 setae (11 + 11 on the first lobe, 4 + 4 on the second, 4 + 4 on the third), this being a characteristic also found in *E. bonadonai* Condé 1979 and *E. pretneri* Condé 1977 (Condé 1977, 1979b). However, in spite of having the same number of setae, the lobes of the genitalia of these three species have a completely different shape and distribution of the setae. *Eukoenenia spelunca* has fusules on moderately dilated processes, as in *E. pauli, E. lawrencei* Rémy 1957 from South Africa and Papua New Guinea, *E. grassii* (Hansen 1901) from South America, and *E. janetscheki* Condé 1993 from Brazil, as discussed by Barranco and Mayoral (2007).

Although the only known individual of *E. spelunca* has a moderately reduced body size (only 720 µm), the value of the bta IV/ti ratio (0.91) is closer to the troglobitic species average (0.95) than to the endogeic species (0.79) (Condé 1996). The value of the propeltidium/bta IV ratio (1.92) suggests prolongation of the appendages, being similar to that of troglobitic species, which is always less than 2 (Condé 1998). Finally, the bta IV is 5.6 times longer than wide at the level of the seta r, being in the range found for cave species, which varies between 3.22 in *E. pretneri* and 10.22 in *E. naxos* (Condé 1998).

The description of a new species of troglobitic Palpigradi for Brazil is very important, keeping in mind the fact that few described species exist not only in the country, but also in the Neotropics region as a whole (Harvey 2003).

Furthermore, in Brazil, the presence of an endemic troglobitic species assures the preservation of the cave in which it was found. Until 2009, all Brazilian caves were protected by law. However, unfortunately, the legislation was altered, and the Brazilian caves now can be destroyed by different anthropogenic activities (especially mining). With the intention of defining which caves can be eliminated and which should be preserved, government officials created categories (based on biological and geological parameters) that define the status of each cave. To assure the preservation of a cave in Brazil, it is necessary, from a biological point of view, that it possesses at least an endemic troglobitic or rare species. Therefore, the description of *E. spelunca*, besides contributing to the knowledge of Palpigradi diversity in the Neotropics, ensures the preservation of a cave and its surroundings.

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SHORT COMMUNICATION

Sheet-web construction by *Melpomene* sp. (Araneae: Agelenidae)

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Abstract. Sheet-webs are built by a variety of unrelated spiders. Some of these spiders are common, but information on their web construction behavior is scarce. This study describes the sheet-web construction behavior of *Melpomene* sp. (Agelenidae) and the sites where webs are built. I recorded the beginning of sheet-web construction by several spiders and analyzed photographs of webs in the field and the laboratory. Web construction consisted basically of two alternating behaviors: laying support threads and the filling in the sheet. These behaviors were repeated during several construction sessions until the available area was filled, or until the web reached approximately 80 cm^2. Apparently the spider uses both ampullate and aciniform lines for web construction, contrary to a recent description.

Keywords: Web building behavior, funnel web, ampullate lines, aciniform lines

Web building behavior in spiders provides useful characters for determining phylogenetic relationships due to its consistency and ease of observation (Eberhard 1982; Coddington 1986; Kuntner et al. 2008), and it is an important aspect of the biology of spiders due to the significance of the web in prey capture. There have been detailed studies of web-building behavior for a number of groups of spiders; however, information is very limited for spiders that build sheet-webs. Furthermore, sheet-weavers include species in distantly related groups of spiders, and their webs differ in structure and types of silk threads used (Griswold et al. 2005). It is very likely that the sheet-web construction behaviors vary among different groups of spiders.

Funnel-web spiders (Agelenidae) construct webs that consist of a flat sheet formed by dense layers of irregularly arranged silk lines near the ground. The sheet is connected to a funnel-shaped tunnel located at the edge or near the middle of the sheet. This tunnel serves as a place to eat, mate, hide and shelter egg sacs (Bristowe 1958; Foelix 1996; Matsumoto 2008). Some webs have threads above the sheet that may serve to intercept flying insects, causing them to fall onto the sheet (Ubick et al. 2005); the importance of this function, however, has not been demonstrated.

The family Agelenidae includes very common spiders like giant house spiders (*Tegenaria duellica*) Simon 1875 and common grass spiders (*Agelenopsis* sp.); nevertheless, details of the sheet-web construction behavior in this family remain unknown. This study provides a description of the sheet-web construction of the poorly studied spider *Melpomene* sp. (O. Pickard-Cambridge 1898) and observations about web placement in its natural environment.

METHODS

I observed the construction behavior of penultimate and antepenultimate females of *Melpomene* sp. collected in the Leonel Oviedo Reserve (1200 m elev.), Universidad de Costa Rica, San José, Costa Rica on April 6–June 30, 2009. Spiders were identified by Darrel Ubick in a previous study (Barrantes & Eberhard 2007). Several adult male and female voucher specimens are deposited in the Museo de Zoología, Universidad de Costa Rica.

Spiders were placed individually in 14 × 14 × 5 cm plastic boxes. The base of each box was covered with black cardboard, pierced by tacks. The tips of the tacks were 5 mm above the surface of the cardboard, and formed a grid with 1.5 cm between tacks. The tacks served as substrates on which the spider built its web, as well as reference points when analyzing the videotapes.

I analyzed the web building behavior of 12 spiders, seven of which had previously built a tunnel inside a twisted or rolled dry leaf. I collected these seven spiders in the field by removing the web around the leaf while the spiders were inside and placed the leaf inside the plastic box. Five other spiders were placed in boxes with two or three dry leaves in which they had not previously made tunnels.

Once inside the boxes, spiders were kept in a dark room with a reverse 12:12 h L:D cycle to facilitate observation of these mainly nocturnal animals. Photographs of the web that had been built were taken every 24 h. The spiders were kept in captivity until the web occupied all available space, or until at least two days passed without further web enlargement (5–12 days). I sprayed the webs with water before taking pictures, in order to reveal the threads of the web. In the case of four randomly selected spiders, I recorded and analyzed the first 90 min of web construction (which began about 5 min after the spiders were placed in the box), using a Sony DCR TRV50 camera in night-shot mode. Because silk threads were not visible in the video recordings, I analyzed the behaviors performed by spiders and not thread placement. I made a diagram of time and behavior location on the plastic box for the spider that wove the largest web area, using Adobe Photoshop CS software. I also analyzed the time that the four spiders spent performing each behavior using JWatcher 1.0. software.

I took photographs of different random areas of one sheet web under a compound microscope to observe the lines placed as the result of each type of spider movement. I also took photographs of 20 sheet-webs in the field to measure their size and compare them with 12 webs built in captivity. I provide a brief description of the sites where spiders built their webs based on my observations while collecting the spiders.

Description of behavioral units.—The construction of the sheet-web consisted basically of three different behaviors: laying support threads, filling in the sheet, and resting /motionless.

Bee Line Movement (BLM): In this behavioral stage, the spider laid the support threads, generally walking fast (almost running) in a straight line without bending or tilting its abdomen, and keeping its posterior lateral spinnerets (PLS) directed posteriorly. Generally it moved in a radial direction from the tunnel or near it, to a substrate (tacks or container wall) beyond the edge of the sheet. When the spider reached the substrate, it flexed its abdomen laterally toward the substrate and paused briefly (0.7 s). During this time the threads were attached to the substrate, probably using the anterior lateral spinnerets. Then the spider returned along nearly the same path to the central part of sheet web or the tunnel.

Sheet Filling Movement (SFM): During this stage of web building behavior, the spider filled the sheet with fine silk. While

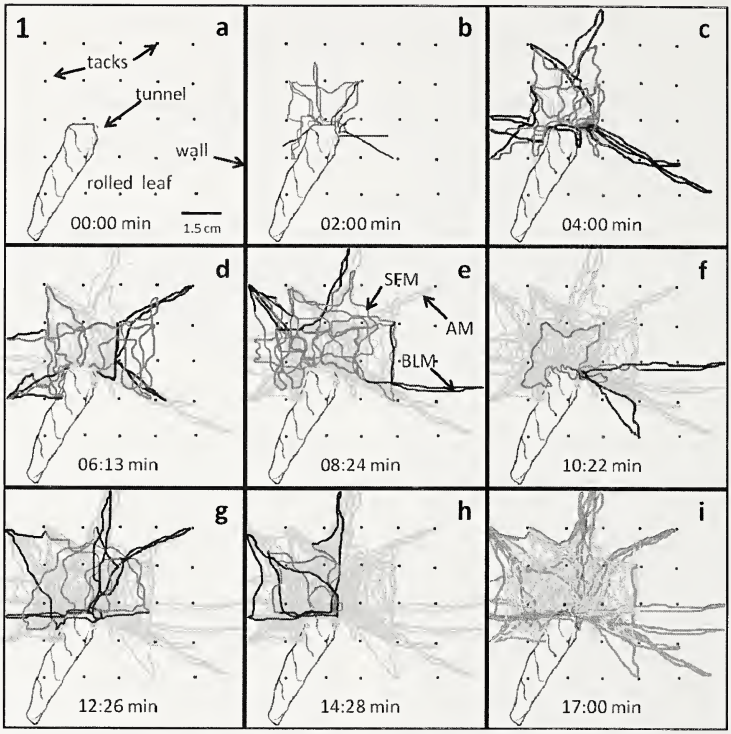

Figure 1.—Path of an individual *Melpomene* sp. during the first 90 min of sheet-web construction. Times shown in each figure indicate the net construction time. a) Before web construction; b–h) paths and types of movements during web construction, coded by color: BLM (Bee-line Movement) = Black, SFM (Sheet Filling Movement) = Dark gray, AM (Accumulated Movements) = Light gray; i) Accumulated construction movements in 90 min observed (darker lines = BLM and lighter lines = SFM). Between g) and h) were 22 min of inactivity; after i) the spider remained inactive.

filling the sheet, the spider walked rapidly, waving its abdomen from side to side repeatedly. Frequently, the PLS were open, forming approximately a 40° angle with the spider's longitudinal axis, while the spider walked and waved its abdomen. During the sheet filling, spiders followed an apparently erratic trajectory (Fig. 1).

Resting/motionless (RM): During this behavior, the spider remained motionless, mainly inside the tunnel or at its entrance.

RESULTS

In the field *Melpomene* sp. built their webs in the leaf litter, on the branches of shrubs, fallen trees and on the trunks of standing trees up to 2 m above the ground. It was common to find aggregations of up to 20 webs in an area as small as approximately 4 m^2. Webs built in the laboratory were similar to those built in the field.

All four spiders that I observed during initial web construction made the same three types of movements, but showed variation in their sequence. These behaviors often alternated (Figs. 1, 2), and their relative durations varied. The spiders repeated BLM many times, forming concentrations of radial threads that supported the sheet-web (Fig. 3b) and gave the exterior border of the web a polygonal shape (Fig. 3a). At least two silk lines were produced during BLM, apparently by the anterior spinnerets (Fig. 3c).

Sometimes the spiders changed from BLM to SFM and vice versa without returning to the tunnel (Fig. 2). SFM occurred mainly in the central zone of the sheet (Fig. 1i) and probably resulted in the addition of multiple layers of silk.

In 1.5 h of web construction recorded, spiders used on average 7.1% (mean = 385 s, n = 4, SD = 223 s) in apparent thread placement: 40.6% (n = 4, SD = 7.8) of this time was spent performing BLM, and 59.4% of the time performing SFM. The rest of the time the spiders were motionless at the entrance or inside the tunnel (approximately 92.9%). During BLM and SFM, the spiders frequently returned to the tunnel entrance; normally they stayed away for approximately 10 s (SD = 14 s). I never observed thread manipulation with the spider legs.

Photographs of webs under the microscope showed at least two types of thread (Fig. 3d). The first type of thread was thick, and was always straight and oriented more or less toward the tunnel. Apparently these thick threads were placed during BLM. The second type of thread was more abundant, thin, often lax, and not oriented in consistent directions as the threads of the first type were. These

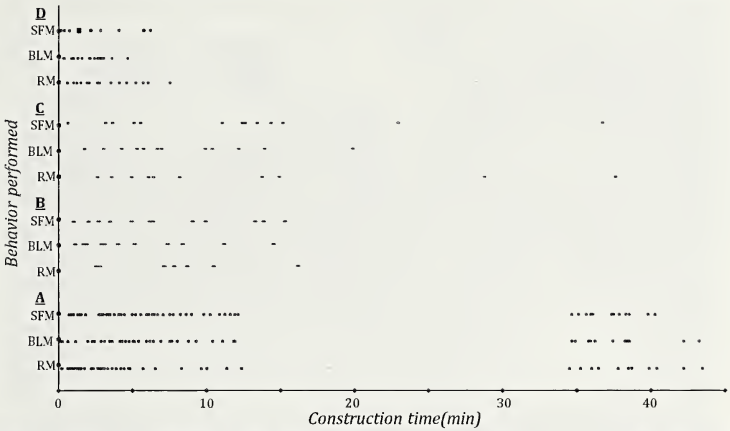

Figure 2.—Behaviors performed by four spiders during the beginning of sheet-web construction. (Spots show when behaviors initiate, not time spent during behaviors). Spider A was also used for Figures 1 and 4.

threads were presumably produced during SFM. I did not observe threads with balls of liquid on them.

Over several days the spider added new web to areas outside the original sheet (Fig. 4), and the sheet sloped more upward at the edges (Fig. 4d), due to the accumulation of attachment points on higher sites on the walls of the box. Areas that were built earlier gradually accumulated a thicker layer of silk. I did not find any order or pattern to where spiders added new web patches. The mean area of sheet-webs in the field was 808 cm^2 (n = 20, SD = 217 cm^2), while that in the laboratory was 110 cm^2 (n = 12, SD = 75 cm^2).

DISCUSSION

The sheet-webs built by *Melpomene* sp. consisted of an irregular, flat area with a tubular retreat. They were composed of non-sticky silk and suspended by silk threads attached at a few points to the substrate. The shape of the sheet web depended on the place of its construction, and the spiders added silk for several days to fill the available space (Blackledge *et al.* 2009).

At least during the first part of construction, and presumably during the remaining process, the construction behavior consisted of two types

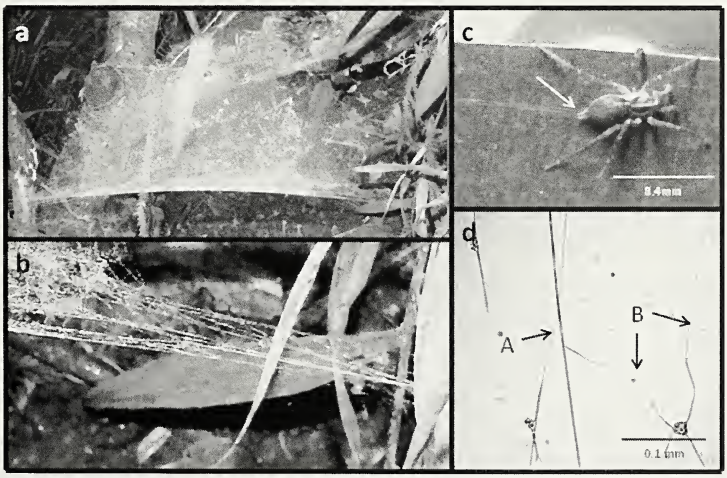

Figure 3.—a) Typical sheet-web of *Melpomene* sp. Note the tunnel in the central upper side. b) Concentration of radial threads that hold the web (detail of the lower right corner of a). c) *Melpomene* sp. during a Bee-line Movement (BLM). At least two threads were produced, and these apparently did not emerge from the posterior lateral spinnerets. d) Silk threads observed at the microscope, A thread probably produced during BLM, B thread probably produced during SFM.

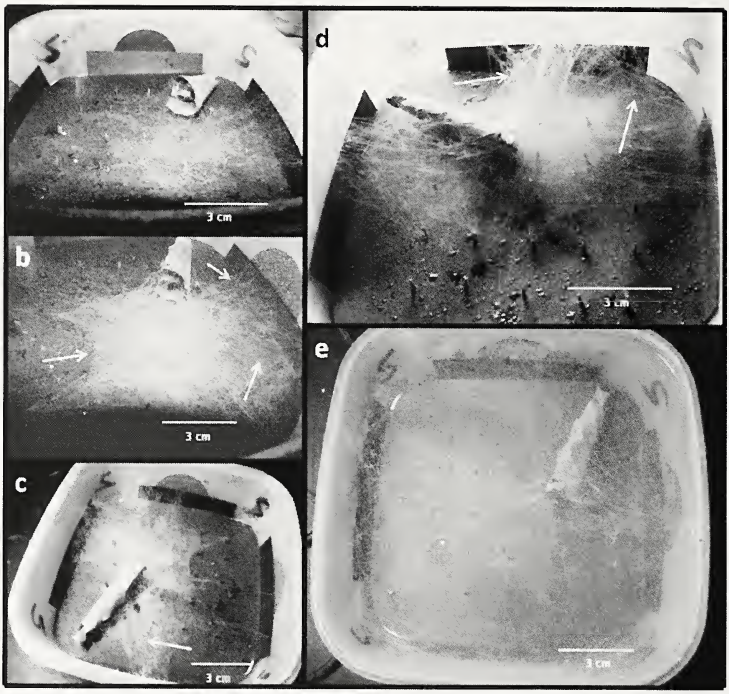

Figure 4.—Gradual development of a sheet web of *Melpomene* sp. a) day 2, b) day 3, c) day 4, d) day 7 (note the slope formation on the sides), e) day 10; finished web. Arrows indicate places where web area increased.

of behavior: placement of supporting threads and placement of filling threads. The support threads were probably produced by the ampullate spigots on the anterior spinnerets and laid during Bee-line Movements. Something similar occurs in *Neoramia*, another agelenid spider that builds a web similar to that of *Melpomene* sp. (Griswold et al. 2005). Ampullate silk probably supports the rest of the web.

During sheet filling movements, the spider repeatedly waved its abdomen with its long posterior lateral spinnerets spread open, and the spider apparently left a swath of silk instead of a single pair of lines as it walked. Griswold et al. (2005) reported that surfaces of the sheet webs of *Euagrus* (Dipluridae) and *Agelenopsis* (Agelenidae) result from the simultaneous action of many aciniform spigots located in the posterior lateral spinnerets. *Neoramia* also has numerous identical spigots in its posterior lateral spinnerets (Griswold et al. 2005). If the arrangement of spigots on the spinnerets of *Neoramia* sp. and *Melpomene* sp. are similar, then the silk laid during sheet filling movements by *Melpomene* sp. is probably also produced by aciniform glands. Unlike those reported by Griswold et al. (2005) in *Euagrus* and *Agelenopsis*, and the report of Blackledge et al. (2009), the web of *Melpomene* sp. also has thicker threads, which has radial orientations.

Barrantes and Eberhard (2007) described how *Melpomene* sp. spreads its posterior lateral spinnerets while wrapping a prey, producing a greater coverage of the silk bands secreted by its long posterior lateral spinnerets. This same increase in coverage is probably also used by this species during the Sheet Filling Movement.

It is well known that when prey falls onto an agelenid sheet-web, the spider grabs it quickly and immediately returns with the prey in a straight line to the tunnel, even if the approach follows a tortuous path, which suggests that the spider uses different cues to calculate the direction toward the tunnel (Mittelstaedt 1985; Görner & Claas 1985; Barth 2002). This ability has been described for orb-web construction of *Leucauge mariana* (Tetragnathidae) (Taczanowski 1881) (Eberhard 1987). Probably similar orientation is important during sheet construction by *Melpomene*, as it continuously returned to the tunnel entrance, suggesting that it knew where it was located. Nonetheless, *Melpomene* sp. spiders might also use the ampullate threads as a cue to return to the tunnel, at least after the web is partially complete, since most have radial orientations. This feature could also be the parameter that the spider uses to obtain its approximate position in the web, though the wandering behavior of experimentally disoriented spiders argues otherwise (Görner & Claas 1985).

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SHORT COMMUNICATION

Suitability of a subcuticular permanent marking technique for scorpions

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Abstract. The primary impediment of long term, high-resolution, ecological studies of scorpions is the difficulty of marking individuals for monitoring and recapture. I tested the use of Visible Implant Elastomer (VIE) as a permanent subcuticular tagging technique in the striped bark scorpion *Centruroides vittatus* (Say 1821). Mortality and prey capture rates of tagged scorpions did not significantly differ from untagged controls. Tag readability was high and comparable to published studies on other arthropod groups. Animals molted (3 treated, 7 control) and gave birth (1 treated, 2 control) successfully. I recommend VIE tagging as a viable solution to what was a major impediment to the proliferation of fine-scale ecological and population-level field research in *C. vittatus* and similar arthropods.

Keywords: *Centruroides vittatus*, mortality, prey capture, tagging, VIE

The primary impediment of long term, high-resolution ecological studies of scorpions is difficulty in marking individuals for monitoring and recapture. Scorpion tagging for ecological investigations has been restricted to various external paints (Sissom et al. 1990). Any external mark used with arthropods is lost with ecdysis. This limits researchers to two forms of long-term study: The first exclusively focuses on adults after their ultimate molt irrespective of immature individuals. This is impractical for species known to undergo postmaturation ecdysis and overlooks younger individuals. The second option is the inclusion of the highly inefficient and precarious practice of maintaining scorpion populations under near-constant observation to allow for the replacement of marks after ecdysis. Subcuticular tags injected just below the epidermal layer should remain within the animal during ecdysis and would therefore be permanent.

Visible Implant Elastomer (also termed Visual Fluorescent Injection Elastomer, or some variant of the two names; hereafter abbreviated as VIE) is a two part silicone-based animal tag injected hypodermically near the body surface as a liquid (Frisch & Hobbs 2006). The injection cures within the animal forming a pliable, biocompatible tag. The ability to read marks noninvasively by visual inspection is a prerequisite for many fine-scale field studies. VIE is highly pigmented in a variety of colors, allowing for visual identification of tags through transparent or semi-transparent material. Combinations of multiple tags in varying colors and injection sites allow for unique identifiers to distinguish tagged groups or individuals from one another. Additionally, commercial VIE is available in a variety of fluorescent colors — a seemingly appropriate attribute for scorpion marking, as ultraviolet light is perhaps the most common collection method for scorpion research.

Visible Implant Elastomer has been used extensively in fisheries management and has gained recent popularity in amphibian tagging. The use of VIE in arthropods has also gained popularity, but only among crustaceans including lobster, shrimp, crab, and crayfish (Claverie & Smith 2007; Pillai et al. 2007; Morgan et al. 2006; Buřič et al. 2008).

Few studies have measured the effects of tagging arthropods (only aquatic Crustacea represented) with VIE against untreated animals. The only report of increased mortality in treated animals compared with untreated controls was among 1.5-mo old *Homarus gammarus* Linnaeus 1758, but no significant difference was found within the same study among seven-month-old conspecifics (Linnane & Mercer 1998). Tag retention rate ranged from 82–100%, and readability ranged from 80–100%, though it should be noted that the dependence of these two measurements has not been addressed in any study reviewed. The most often noted concerns were errors in interpreting tag color (Curtis 2006) and, in a few cases, minor tag migration (Davis et al. 2004; Woods & James 2003) or fragmentation (Clark & Kershner 2006; Linnane & Mercer 1998). Two studies successfully injected particularly small specimens with mean weights (± SD) of 1.25 ± 0.5 g and 0.9 ± 0.8 g (Jerry et al. 2001; Pillai et al. 2007). These were also the only studies to show reductions in tag retention, though minor. Animals successfully molted while retaining tags during all studies reviewed.

No study of the use of VIE tagging with arachnids has been published. A different subcuticular mark, Passive Integrated Transponder (PIT) tags (a radio frequency identification technique) has been tested successfully in three large Theraphosidae species (Reichling & Tabaka 2001). Though the development of smaller (12.5 × 2.1 mm, 0.102 g) PIT tags in recent years has allowed for implantation of these devises in smaller animals, PIT tags can only physically fit in the largest arthropods. In addition to PIT tags, coded wire tags and visual implant alphanumeric tags were considered. Relative to the above tagging techniques, VIE is cost-effective with a minimally invasive application procedure, should impose minimal disruption to normal animal functioning, can be implemented on very small animals, and is not lost with ecdysis.

I here test the hypothesis that VIE tagging would not increase mortality or decrease prey capture in the terrestrial arthropod *Centruroides vittatus* (Say 1821).

METHODS

This study required a readily available scorpion species of moderate size. *C. vittatus* is locally abundant and is of moderate size, thereby increasing this study's range of inference for future field research. I included juvenile *C. vittatus* in the study to further demonstrate that VIE tagging can be used in small individuals and those that undergo ecdysis.

Colleagues and I collected *Centruroides vittatus* from Jeff Davis, Garza, and Randall Co., Texas, USA, on 26 September–22 November 2009. Each specimen was housed in a 16 oz (11.5 cm × 8 cm diam.) clear polyethylene container with a thin (ca 1 cm) layer of commercially purchased sand and a crumpled white paper towel to increase enclosure complexity and provide retreats. Small holes were put in the container's sides for ventilation. Containers were stored in an incubator averaging 28.3 ± 0.1° C SD and 30 ± 1.4% humidity.

Captive-bred house crickets (*Acheta domestica* (Linnaeus)) were offered to scorpions weekly and removed if not consumed after all other scorpions had fed (duration mean: 49 ± 13 min SD). The side of

each container was sprayed with tap water after prey capture to increase humidity and allow drinking from droplets.

I required collected scorpions to meet two criteria before being included in the study: Each individual had to survive in captivity for one month and capture prey within that time. I weighed animals meeting these criteria with an electronic scale (instrument error ± 0.01 g) and measured midline carapace length and mesosomal length with calipers (instrument error ± 0.2 mm). Scorpions included in the study had a mean ± SD weight of 0.34 ± 0.18 g, midline carapace length of 4.15 ± 0.69 mm, and mesosomal length of 13.84 ± 3.14 mm. The smallest animal weighed 0.07 g, had a 2.2 mm midline carapace length, and a 9.1 mm mesosoma length. Animals were randomly assigned to two equal groups. One group was randomly assigned for treatment by injection with VIE (n = 23; 8 males, 13 females, 2 juveniles) and the other acted as the study's untreated control (n = 23; 9 males, 10 females, 4 juveniles). I injected commercial red fluorescent VIE (Northwest Marine Technology™, Inc., Shaw Island, Washington, USA) dorsally through the posterior membrane of one of four randomly selected tergites using a 28 gauge, 0.3 cc syringe with a 13 mm beveled needle (BD™, Franklin Lakes, New Jersey, USA). This resulted in a longitudinal line of VIE positioned dorsolaterally just inferior to the cuticle. This location avoids the dorsal heart while maintaining tag readability. I followed a recommendation made by Godin et al. (1996) to position the tag parallel to muscle striation to avoid undue scarring and inflammation. I recorded the time (rounded to the nearest min) it took for each group to feed after injection.

I monitored treatment and control groups for 3 mo after tag implantation. I recorded if each individual captured prey during each feeding session. I also noted births, deaths, and ecdysis events. Volunteers inexperienced with reading VIE tags independently completed a test to determine tag readability (tag presence and placement) using ultraviolet light.

I totaled deaths in both groups at the study's end and performed a chi-square goodness of fit test to test for a difference in mortality between treated and control groups. I used Mann-Whitney U Rank Sum tests to determine if there was a significant difference in prey capture latency between treatment and control groups right after the tagging procedure, and over the entire study period. I conducted a Mann-Whitney U test concerning potential seeondary variables that might have caused experimental error: mean animal weights, carapace lengths, and mesosomal lengths of each group. All statistics had an α value of 0.05.

RESULTS

Mortality of tagged individuals was not significantly greater than controls (10 and 9 individuals; $\chi^2_1 = 0.053$, $P = 0.818$). No treated animals died immediately after the injection procedure. Four control (17.4%) and five treated (21.7%) scorpions did not capture prey immediately after the injection procedure. Among scorpions that did feed, treated animals took a significantly longer time to capture prey (mean ± SD = 11.9 ± 26.7 min) than controls offered prey during the same feeding session (mean ± SD = 6.1 ± 8.1 min; $U_{19,18} = 94.50$, $P = 0.020$). The relative frequency of treated and control animals that captured prey during the feeding sessions was not significantly different ($U_{12} = 64.00$, $P = 0.664$). There was no significant difference in weight, carapace length, or mesosomal length between treated and control scorpions ($U_{23} = 195.00$, $P = 0.129$; $t_{44} = -2.002$, $P = 0.051$; $U_{23} = 188.50$, $P = 0.097$).

Two assistants observed twenty-three animals to test readability. Of 46 observations, only one resulted in a tagged animal identified as untagged (98% correct presence/absence observations). Three animals were identified with tags but incorrect tag placement, accounting for five misidentifications (89% correct placement observations) with both assistants misidentifying two of the same animals. During the study three treated and seven control animals molted and two tagged and one control animal gave birth. No patterns were found between these events and mortality or readability.

DISCUSSION

Survivorship of tagged animals did not significantly differ from the control group and was similar to those reported for other arthropods (Clark & Kershner 2006; Mazlum 2007; Claverie & Smith 2007; Pillai et al. 2007). Delay in prey capture among tagged animals was not surprising. It is reasonable to expect that animals handled and injected would exhibit delayed prey capture. Despite this result, mortality and feeding frequency did not differ between groups. While some short-term behavioral changes may result from the tagging procedure, this study found no evidence of any long-term impact of VIE injection. The three tagged animals that molted and one that gave birth did so successfully.

Tag readability was high, and within the 80–100% range indicated in studies of other arthropod groups. Assistants showed high consistency in tag identification. Both assistants made the same incorrect tag presence/absence determination, and two of the three same tag location misreads. This seems to indicate that the tagging procedure was to blame for misreads, and readability could near 100% with improved methods. Readability seemed to increase with experience in the VIE injection procedure. For this reason, I recommend practicing on preserved specimens and limiting the injection procedure to researchers with tagging experience.

Readability was not enhanced by the use of an ultraviolet light. Several commercially available VIE colors – including the red color used in this study – fluoresce brightly under ultraviolet light. When injected under scorpion cuticle, ultraviolet light induced the otherwise translucent cuticle to fluoresce, thereby obscuring the tag. Field researchers should read tags under white light, not ultraviolet. It should also be noted that VIE is not suitable for scorpion species with highly pigmented cuticle that will obscure tags.

I chose four dorsal mesosomal tagging locations because I postulated VIE in this area would impact the animals least. Tagging the metasomal segments or the trochanter, femur, or patella leg segments may result in slightly higher readability without increased mortality, but these locations have not yet been tested. More importantly, these alternate locations would increase the number of unique marks from 256 marks using four colors with the four locations tested in this study, to 5376 when also marking five metasomal segments – a number more than sufficient for long-term studies.

These results indicate that VIE is a suitable tagging alternative to traditional external marks in *Centruroides vittatus*. This study should encourage the proliferation of fine-scale ecological and population-level field research of terrestrial arthropods.

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SHORT COMMUNICATION

Female attack is not necessary for male copulatory organ breakage in the sexually cannibalistic spider *Argiope argentata* (Araneae: Araneidae)

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Abstract. In sexually cannibalistic spiders, males usually only copulate with one female. This selects for male strategies to improve paternity success in their single mate. Male mating strategies can include genital damage during female attack in some cannibalistic orb-weaving spiders, where males are dwarf and females polyandrous. We explored whether sexual cannibalism is necessary for male genital damage in the silver spider *Argiope argentata* (Fabricius 1775) by performing mating trials with recently killed virgin females. We found that males can break off their copulatory organs without female intervention and spontaneously die during copulation. Results suggest that genital damage evolved in response to sperm competition in this species.

Keywords: Genital damage, sperm competition, mating plug

Sexual cannibalism, defined as instances where females kill and consume conspecific males before, during, or after copulation, has been considered an extreme case of conflict of interest between the sexes (Elgar 1992). Males benefit by fertilizing more eggs, while females can benefit by remating (Simmons 2005), leading to sexual antagonistic coevolution (Arnqvist & Rowe 2005). Sexual cannibalism has been reported for a variety of invertebrates, including crustaceans, insects, and arachnids, and is particularly frequent among spiders (Elgar 1992). If males transfer sperm successfully, then sexual cannibalism may be part of a male mating strategy (Elgar & Schneider 2004). In these cases, males maximize their paternity in an act of single mating, becoming monogynous. Such terminal investment without parental care has been shown to evolve under a male-biased effective sex ratio with high risk of sperm competition (Fromhage et al. 2005).

In a framework of high sperm competition, males will develop offensive and defensive strategies to protect paternity, including the use of mating plugs. Mating plugs can be substances that become hard while occluding genital ducts (Baer et al. 2001; Polak et al. 2001; Aisenberg & Eberhard 2009), or parts or the entire male copulatory organ, a process known as genital damage (Eberhard 1985; Kamimura 2003; Uhl et al. 2010). Genital damage is widespread among sexually cannibalistic spiders, where males usually break off parts or the entire copulatory organ during copulation (Andrade 1996; Andrade & Banta 2002; Elgar & Schneider 2004; Foellmer & Fairbairn 2004; Miller 2007; Nessler et al. 2008). Male spiders' copulatory organs are paired (palpal bulbs) and the intromittant features, the emboli, are introduced into the paired female genital openings during mating, usually one at a time. In spiders, all known cases of genital damage occur in entelegyne spiders in which the genitalia are sclerotized and the ovipository duct is independent from the copulatory ducts, and therefore not occluded by mating plugs (Uhl et al. 2010).

In spiders, paternity success is usually linked to copulation duration and sperm transfer (Elgar 1995). Schneider et al. (2006) and Nessler et al. (2006) suggested the occurrence of a sexual conflict over copulation duration in the orb-web spider *Argiope bruennichi* (Scopoli 1772), where female attack occurs precisely when the male dislodges the used copulatory organ and tries to insert the other one (Schneider et al. 2006). In the orb-web spider *Nephila plumipes* (Latreille 1804), palpal organ breakage increases male survivorship, allowing a second insertion and increased copulation duration, whereas males that do not break their organs are cannibalized (Schneider et al. 2001). In *Argiope lobata* (Pallas 1772), cannibalized males break off their genital copulatory organs more frequently than surviving males, suggesting that sexual cannibalism facilitates genital damage (Nessler et al. 2008).

The silver spider *Argiope argentata* (Fabricius 1775) is an araneid spider with Pan-American distribution (Levi 1968). In the field, Robinson & Robinson (1980) observed that males arrive on females' webs, court from the periphery, and afterwards move to the hub of the web where mating usually occurs, and sexual cannibalism always occurs during or after copulation. In the laboratory, virgin females are usually receptive to courting males, but they attack them during the first insertion, forcibly dislodging males from their genitalia with their third pair of legs, resulting in 70% of the males dying (Ghione 2008). Surviving males reinitiate courtship and perform a second insertion; after that they are consumed by the females. Males always break the apical region of the embolus, including a singular sclerite or spur of unknown function (Levi 1968), that remains stuck inside the female ducts (Ghione et al. 2006; Ghione 2008).

In the present study, we used freshly killed females to explore experimentally if males can break off their copulatory organs by themselves, or if female cannibalistic attack determines male genital damage. We hypothesize that the female's attack has a direct impact on the copulatory outcome, both in the removal of the male and the breaking off of the inserted copulatory organ.

We collected nine subadult males, 11 subadult females, and 18 adult males of *A. argentata* between September to March (spring and summer of 2005–2006 and 2006–2007), in meadows at Piedras de Afilar, Canelones, Uruguay (34°45′42.5″S and 55°33′10.8″W), a temperate region. We housed spiders individually in 500-ml glass containers, providing water daily, and *Tenebrio* sp. larvae (Tenebrionidae) twice per week.

In order to determine if the male can break off his copulatory organs by himself or if it is the female that breaks them off when she abruptly removes the male from her genitalia, we carried out experiments with recently killed virgin females. We killed them by means of hypothermia, placing them at a low temperature for 20 minutes. Afterwards, dead females were carefully attached "face down" (typical "sit and wait" and mating posture) to their own web's radii, fixed by adding melted paraffin onto each of their eight leg tarsi. Once females were fixed to the web in the proper position, we

Figure 1.—a) Dead male attached to female genitalia in *Argiope argentata*; b) the same male inserting the right palpal organ and showing the left one with the broken embolus tip (arrow).

carefully removed silk with forceps from their spinnerets and added it to the hub or center of the web, as naturally occurs when females are positioned in the web.

For each trial, two adult males were carefully placed equidistantly from the margin of the orb-web of a recently attached killed female. We placed two males to increase the likelihood of copulation. We simulated female behaviors observed in a sexual context (Ghione, pers. obs.) by softly shaking the web, using a pencil to prod the female's corpse, in response to male courtship. We replaced males when a male did not court during a 15-min period, a male courted from the periphery but did not move to the hub within one hour, or a male initiated courtship but did not proceed to copulation over the course of two hours. This exchange of unsuccessful males continued until either copulation occurred or a total of 5 hours had elapsed, limited by the rapid decay of the dead female in warm experimental conditions. When one male achieved copulation, the other male was immediately (but carefully) removed from the web in order to avoid male-male interferences.

We performed a total of 11 trials and obtained four successful copulations. The occurrence of copulations was highly unpredictable

and difficult. Male genital damage was determined under a dissecting microscope. We deposited voucher specimens in the arachnological collection of Facultad de Ciencias, Montevideo, Uruguay.

In all the 11 trials, males courted the females and responded to the simulations of female sexual behaviors. All copulating males died immediately after copulation. Three males performed two palpal insertions. They jumped off the female epigynum a few seconds after their first insertion and escaped from the female web, but immediately returned and courted again. After their second insertion, all three died spontaneously, remaining attached to female genitalia. One male died spontaneously after performing a single palpal insertion. We did not know the exact time of death, but death was confirmed after we proceeded to carefully touch unmoving males (in mating position) with a candy pin after an arbitrary period of 30 minutes of immobility. Each of the three males that performed two insertions broke off the first inserted organ and the embolus tip remained inside the female reproductive tract. The second inserted copulatory organs were not broken off due to males dying and remained connected to female epigynum (Fig. 1).

Results indicate that males spontaneously die in copula, evidenced by the absence of female intervention, as was observed for other *Argiope* species (*A. aurantia* [Lucas 1833]: Foellmer & Fairbairn 2003; *A. aemula* [Walckenaer 1841]: Sasaki & Iwahashi 1995; *A. keyserlingi* Karsch 1878: Herberstein et al. 2005; *A. bruennichi*: Schneider et al. 2006). In entelegyne spiders, the hematodochae expands during copulation, allowing the penetration of the embolus into the female genital tract (Foelix 1996). In *A. argentata*, the expansion of the haematodochae probably requires sequestration of a large percentage of hemolymph from body circulation, provoking males' deaths. The single male which died after his first insertion could possibly have mated previously in the field. However, this male was previously examined under the dissecting microscope, and possessed both intromittant organs intact. Therefore, a previous mating in the wild of this individual is improbable.

Three males were able to disengage and break off their copulatory organs without female intervention, contrary to our prediction. Our results confirm that males alone engage in genital autotomy; female action during cannibalism is not required. This is the first demonstration of voluntary emasculation by males during copulation in *Argiope* species. Each male of *A. argentata* did not dislodge himself from the epigynum after the second insertion and died firmly attached to female genitalia, suggesting that the entire male body could act as a whole-body mating plug, as was stated by Foellmer & Fairbairn (2003) for *Argiope aurantia*. In *A. argentata*, the expanded copulatory organ could continue ejaculating seminal fluids after the male's death, as was indicated by Knoflach & van Harten (2001) for theridiid spiders. Interestingly, males would be impeded from remaining attached to the genitalia if the female was alive.

In the present study, we found that males of *A. argentata* can voluntary break off their genital organs, suggesting that there is no obligate relationship between genital damage and sexual cannibalism in this species. This suggests that sperm competition would be the sexual selection mechanism that underlies this particular behavior of voluntary genital mutilation. Male monogyny has been stated to evolve under a male-biased effective sex ratio (Fromhage et al. 2005), favoring extreme male strategies to ensure paternity. Nevertheless, an increased sample size and experiments with other modifications interfering with sexual cannibalism would help to confirm this hypothesis in this spider.

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SHORT COMMUNICATION

Predatory interactions between *Centruroides* scorpions and the tarantula *Brachypelma vagans*

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Abstract. In the Yucatan Peninsula, the tarantula *Brachypelma vagans* Ausserer 1875 is commonly associated with human settlements, as are the scorpions *Centruroides gracilis* Latreille 1804 and *C. ochraceus* Pocock 1898. Nonetheless, scorpions are virtually absent from villages showing a high density of tarantulas. Predatory interactions between these predators could explain the lack of local overlap. To test this hypothesis, we observed the behavioral interactions between *B. vagans* and *C. gracilis* or *C. ochraceus* in experimentally controlled conditions, and we compared these interactions to interactions between the tarantula and two prey species: cricket and cockroach. For observations, a pre-adult tarantula was placed in an experimental arena in which we introduced either a scorpion or an insect. In all, 115 trials were performed. We recorded time elapsed and behavioral responses: avoidance, attack, escape, capture, and attack success. Tarantulas preyed on all prey with the same attack success (63.8% ± 0.8%), but they attacked and captured cockroaches quicker and more often than the other prey (87% vs. 50%, and 57% vs. 30%, respectively). Scorpions attacked tarantulas in 25.5% of occasions, but they were never successful, and were killed in 9% of occasions. We conclude that tarantulas are potential predators of scorpions. Moreover, in villages where tarantulas are abundant they might prevent the presence of scorpions. Thus the presence of this non-aggressive tarantula may be beneficial from the human perspective.

Keywords: *Centruroides ochraceus*, *Centruroides gracilis*, cockroach, cricket, Yucatan Peninsula

The Mexican redrump tarantula, *Brachypelma vagans* Ausserer 1875 (Araneae: Theraphosidae), is distributed from Mexico to Costa Rica, and is also present in Florida (Valerio 1980; Edwards & Hibbard 1999). Despite its large range, most of its natural history is poorly known (but see Machkour-M'Rabet et al. 2005, 2007), particularly its predatory behavior, but for two studies describing cannibalism in the species (Hénaut & Machkour-M'Rabet 2005; Dor et al. 2008). One previous study by Marshall (1996) reported that free-ranging *Brachypelma* spiders are nocturnal and feed on ground-dwelling arthropods, and possibly on small vertebrates. It is also known how sensory channels are involved in prey detection in tarantulas (Blein et al. 1996).

Brachypelma vagans habits are similar to those of scorpions as sit-and-wait nocturnal predators (Hadley 1974; Skutelsky 1995; Pinkus-Rendón et al. 1999), except that *B. vagans*' predatory activities occur within or near the burrow. These burrows can be very densely distributed, as was found in rural settlements of the southern Yucatan (Machkour-M'Rabet et al. 2007). Like *B. vagans*, scorpions in the Yucatan Peninsula are commonly found in or around houses, where 80% of scorpion stings occur (Pinkus-Rendón et al. 1999). Therefore, tarantulas and scorpions are probably competitors, as well as each other's predators, in urban settings.

In the southern Yucatan, two scorpion species, *Centruroides ochraceus* Pocock 1898 (Scorpiones: Buthidae), locally called "yellow scorpion", and *Centruroides gracilis* Latreille 1804 (Scorpiones: Buthidae), locally called "black scorpion", regularly appear in houses and backyards. Our personal observations over several years indicate that approximately ten scorpions are found per house per year. The sting of *Centuroides* scorpions from Yucatan is rarely a source of complications for humans, and only a local reaction usually occurs (Pinkus-Rendón et al. 1999). However, peri-domestic scorpions in Mexico represent a real health problem, with more human deaths annually than in any other country (Ramsey et al. 2002).

Previous casual observations of scorpions in rural villages showed that anywhere that tarantulas are present, scorpions are absent, even if they are found in the surroundings of the villages (Y. Hénaut pers. observ., 2005–08). These observations were confirmed by local people in several villages of the southern Yucatan (A. Dor; S. Calmé, pers. observ.), including those where Machkour-M'Rabet et al. (2005, 2007) found high densities of *B. vagans*. We hypothesized that the absence of scorpions in areas of high density of tarantulas may be the result of predation of *B. vagans* upon scorpions. Spiders and scorpions might be involved in intra-guild predation relationships, as observed for the wolf spider *Schizocosa avida* Walckenaer 1838 with the scorpion *Centruroides vittatus* Say 1821 (Punzo 1997), and for the Mediterranean tarantula *Lycosa tarantula* Linnaeus 1758 with the Occitan scorpion *Buthus occitanus* Amoreux 1789 (Moya-Laraño et al. 2003; Williams et al. 2006).

In this paper, we test the hypothesis that the larger red rump tarantula successfully preys on scorpions by experimentally pairing individuals of *B. vagans* with individuals of the scorpion species *C. ochraceus* and *C. gracilis*, and recording the behavioral response of both arthropods. Additionally, we observed the predatory behavior of *B. vagans* upon two common prey insects, which provided a basis for comparison.

METHODS

Field observations.—We assessed the spatial segregation between tarantulas and scorpions by recording sporadically the presence of scorpions and tarantulas in several areas of the southern Yucatan: Calakmul Biosphere Reserve nucleus area, three villages (11 de Mayo, Zoh-Laguna and Raudales) and

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the city of Chetumal on January 2005–September 2007. In daylight, we actively searched resident tarantulas (occupying a burrow), errant adult tarantulas, juvenile tarantulas (body size ≤ 1.0 cm) and scorpions, to record their presence. We searched underneath stones, fallen trunks, and into burrows. Second, we interviewed local people about the presence of the organisms of interest. Because of the nature of these data, no statistical analysis could be performed.

Collection and care of arthropods.—We collected 25 individuals of black scorpions, 30 individuals each of yellow scorpions, crickets and cockroaches, and eleven pre-adult tarantulas. We reared the latter in the laboratory from several days to several weeks on June 2005–January 2006. All arthropods were maintained under the following laboratory conditions: one individual per plastic cylinder (13 cm diam. × 5 cm height) containing a cup filled with water to keep the humidity high. Water was changed weekly. Room temperature was maintained at 26° C, similar to natural conditions. Spiders were fed with *Zophobas morio* (Coleoptera: Tenebrionidae) larvae. All voucher specimens are deposited in the Collection of the Museum of Zoology of El Colegio de la Frontera Sur, Chetumal, Quintana Roo, Mexico.

Interaction trials.—Besides tarantulas (*B. vagans*) and the two aforementioned species of scorpions (*C. ochraceus* and *C. gracilis*), the arthropods used during the experiments were cockroaches (*Periplaneta americana*) and crickets (*Acheta domesticus*). Body size was determined by measuring the distance from the extreme anterior point of the prosoma (arachnids) or the head (insects) to the hindmost part of the opisthosoma (arachnids) or abdomen (insects). These distances were measured for a sample of each group of arthropods to ensure that prey were of comparable size: crickets (1.98 ± 0.16 cm, $n = 20$), cockroaches (2.08 ± 0.31 cm, $n = 20$), yellow scorpions (2.60 ± 0.18 cm, $n = 30$, and black scorpions (2.68 ± 0.04 cm, $n = 25$). Tarantulas had a mean size of 3.45 ± 0.43 cm ($n = 11$), which was significantly larger than individuals of both scorpion species (Mann Whitney U test: yellow scorpion $U = 2.89$, $df = 1$, $P < 0.01$; black scorpion $U = -4.33$, $df = 1$, $P < 0.001$).

Each tarantula/prey encounter was repeated 30 times, except in the case of black scorpions, for which there were 25 repetitions. All individuals were used once, except tarantulas, since only 11 were available; thus, each tarantula was used about 10 times. Before any trial, all tarantulas were starved for two weeks and randomly paired with a prey item. As soon as an encounter was finished, the tarantula was removed from the arena and starved again if it succeeded in catching and eating the prey. Otherwise, the tarantula was fed with *Zophobas morio* before being starved. Because of the time elapsed between repetitions using the same tarantula (≥ 14 da), each trial was considered independent with respect to any change that could come from experience. The whole experiment lasted 6 mo. All the predation experiments were conducted in plastic boxes (29.5 cm width × 44 cm length × 23.5 cm height). A tarantula was released into the box, and after one minute, the second individual was introduced approximately 10 cm from the spider. Each experimental trial occurred for a maximum duration of 30 min or finished when an arthropod was captured. During the trials we characterized the motion behavior of the second individual before it met the tarantula as follows: quick, slow, or immobile.

We recorded the following behaviors for both arthropods during the trials: 1) Avoidance: when an individual kept its distance from the other following a tentative approach of the latter; 2) Attack: if an individual moved quickly toward the other and made contact with it; 3) Capture: when an individual was bitten or stung after an attack; 4) Escape: when an individual ran away from the other after the latter attacked, without having been bitten or stung; 5) Non-agonistic behaviors (NAB), such as no activity or no movement, which were recorded and classified as a single category. Based on the frequencies of behaviors, we constructed flow diagrams. We also estimated the attack success of tarantulas as being the number of successful captures divided by the number of attacks. The latency before an attack was recorded from the time the second individual was introduced into the experimental arena until the attack occurred.

Data analysis.—The frequencies of avoidance, attack, capture were compared by log likelihood tests (G test) among the four types of encounters (tarantula vs. cockroach, tarantula vs. cricket, tarantula vs. yellow scorpion, and tarantula vs. black scorpion). The frequencies of trials ending with the capture of the individual that first attacked (reverse fate), and attack success (the proportion of prey attacked actually captured) were also analyzed using G tests. Latencies before attack were compared among the four types of encounters using a multiple comparisons Kruskal-Wallis test.

RESULTS

Confirming our previous aneedotal observations, active searches in the field and interviews indieated that scorpions were absent locally when burrowing tarantulas were present. However, the presence of errant adult or juvenile (body size ≤ 10 mm) tarantulas did not prevent the presence of scorpions (Table 1).

The interactions we provoked experimentally between tarantulas and scorpions differed from those of tarantulas with insects mainly because both scorpions and tarantulas were capable of attacking each other, whereas crickets and cockroaches never attacked tarantulas (Fig. 1). The attack behavior of both scorpion species toward a tarantula was similar ($G = 0.05$, $df = 1$, $P = 0.82$), and tarantulas behaved similarly regardless of the scorpion species ($G = 0.002$, $df = 1$, $P = 0.96$). However, the frequency of attacks of tarantulas on scorpions was significantly higher than that of scorpions on tarantulas (43.6% vs. 25.5%, respectively: $G = 4.06$, $df = 1$, $P = 0.04$).

Another main difference between the four types of encounters was the lower number of non-antagonistic behaviors (NAB) during the interactions between cockroach and tarantula ($G = 10.00$, $df = 3$, $P = 0.01$). Tarantulas presented NAB in only 7% of encounters with cockroaches, compared with more than 30% for the confrontations with scorpions or crickets. Furthermore, the frequency of attacks was much higher for tarantula-cockroach interactions than for any other of the three interaction types ($G = 6.50$, $df = 3$, $P < 0.001$), with 87% of tarantula attacks on cockroaches compared with less than 55% on scorpions or crickets. Attack latency was similar for all prey (Kruskal-Wallis test: $H = 5.47$, $n = 42$, $df = 3$, $P = 0.14$), even if cockroaches were attacked more quickly (191 ± 61 s) than the other prey (cricket: 450 ±

Table 1.—Presence (+) and absence (−) of scorpions and tarantulas in several sites of the Southern Yucatán (11M: 11 de Mayo; R: Raudales; ZL: Zoh-Laguna), according to the status of tarantulas (resident, errant or juvenile) and data source (interview or active research).

Data source	Coordinates	Site	Scorpion	Resident tarantula	Errant tarantula	Juvenile tarantula
Interviews	18°29′58.73″N 88°18′09.54″W	Chetumal - South	+	−	−	−
	18°30′08.72″N 88°17′03.13″W	Chetumal - East	+	−	−	−
	18°32′48.69″N 88°16′16.67″W	Chetumal - North	+	−	+	−
	18°07′21.00″N 89°46′59.98″W	Calakmul	+	−	−	+
Active research	18°06′59.90″N 89°27′39.76″W	11M - Secondary forest	+	−	+	−
	18°42′35.32″N 88°15′20.44″W	R - Dirt track side	+	−	+	+
	18°42′27.12″N 88°15′21.74″W	R - Backyard	−	+	+	+
	18°35′24.06″N 89°24′59.09″W	ZL - 2 Houses and backyard	−	+	+	+
	18°05′27.55″N 89°27′38.15″W	11M - Backyard	−	+	+	+
	18°05′26.06″N 89°27′38.11″W	11M - Football camp	−	+	+	+

135 s; black scorpion: 567 ± 300 s; yellow scorpion: 570 ± 286 s). Cockroaches were the only prey to move constantly and quickly when introduced in the box, whereas tarantulas, crickets and both scorpion species stayed mainly immobile. Cockroaches were also the only prey that showed avoidance.

After an attack, the individual under attack (prey) could be captured or could escape, as was generally observed for insects, or might even attack in return, as observed with tarantulas when they were first attacked by scorpions. The frequencies of escape behavior, based on the number of attacks by tarantulas, were similar among the four types of interactions ($G = 2.00$, $df = 3$, $P = 0.50$), with a tendency for the cockroach to escape more often. However, when a scorpion attacked, the tarantula almost never tried to escape (0% and 4% when attacked by yellow and black scorpions, respectively).

All captures were realized by tarantulas, without regard for the species they confronted. In other words, even when a scorpion attacked a tarantula, if none of the individuals escaped, the issue was always a win for the tarantula. However, the efficacy of tarantulas varied with the potential prey. The frequency of captures was higher with cockroaches

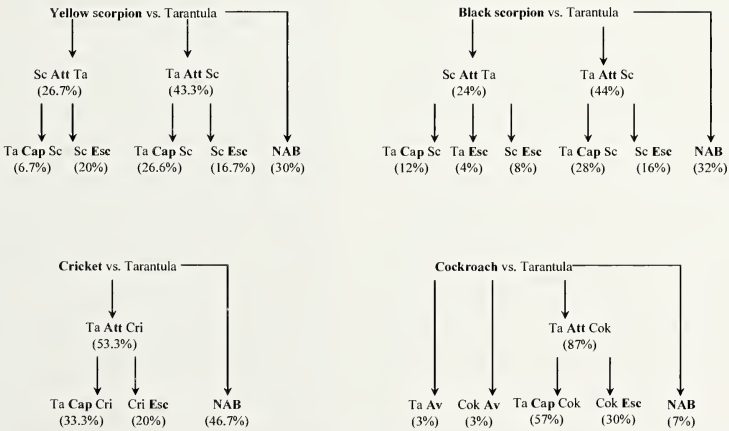

Figure 1.—Flow diagrams of the predation sequence of tarantulas (Ta) on yellow and black scorpions (Sc), crickets (Cri), and cockroaches (Cok). Behaviors as follows: non-antagonist behavior (NAB), avoidance (Av), attack (Att), escape (Esc) and capture (Cap). The sum of percentages at the bottom of each diagram equals 100%.

(half of the trials) than with crickets or scorpions (about one third of the trials) ($G = 16.50$, $df = 2$, $P < 0.001$). Nevertheless, the frequency of successful attacks by tarantulas was similar for all prey (yellow scorpion: 61.5%, black scorpion: 63.6%, cricket: 62.5%, cockroach: 65.5%; $G = 0.06$, $df = 3$, $P = 0.90$).

DISCUSSION

In laboratory conditions, we showed intraguild aggressive behavior between scorpions of two species, *Centruroides ochraceus* and *C. gracilis*, and *Brachypelma vagans* tarantulas. However, predation was only carried out by tarantulas, regardless of which species attacked first. Moreover, in response to an attack by a tarantula, scorpions' defense capabilities were not more effective than those of cockroaches or crickets. This predatory relationship between scorpions and tarantulas contrasts with that reported in previous studies, in which scorpions were predators of spiders (Polis & McCormick 1986; Punzo 1997; Moya-Laraño et al. 2003; Williams et al. 2006). In these earlier studies, however, scorpions were larger than spiders (Punzo 1997; Williams et al. 2006), whereas our experiment involved spiders that were larger than scorpions, with an inverse predation interaction. Body length is undoubtedly a critical factor accounting for the conflicting results of the interactions between these predators. As a matter of fact, in the context of intraguild predation, Polis et al. (1989) demonstrated that predation interaction could be mutual and was size dependent, with the larger individuals of any species always preying on smaller individuals of the other species.

This work offers the first description of this tarantula's interaction with prey, and allows us to conclude that *B. vagans* tarantulas have extensive capabilities of prey capture. *Brachypelma vagans* attacked the three types of prey offered to it, namely *Centruroides* scorpions, crickets, and cockroaches with similar success. Tarantulas, however, attacked and captured cockroaches more often than crickets or scorpions. This advantage was certainly related to the capacity of tarantulas to detect prey vibrations (Blein et al. 1996), as cockroaches were very active and mobile.

In peri-domestic environments where tarantulas are numerous, their ability to prey on scorpions may explain the lack of scorpions (as observed by the authors), even if these are considered common in this kind of environment (Pinkus-Rendón et al. 1999). It is noteworthy that only the presence of adult, resident tarantulas (occupying a burrow) was related to the absence of scorpions. Therefore, based on our laboratory observations, we hypothesize that spatial distribution of scorpions is limited by predation risk by adult resident tarantulas.

The presence of tarantulas in backyards might actually prove to be a good way to avoid scorpion intrusion into houses, and be used as an argument to protect these spiders. From the human perspective, *B. vagans* is less dangerous than scorpions, because it is not aggressive (Locht et al. 1999), its bite is harmless and not very painful (Hénaut et al. 2006), and it does not invade houses as scorpions do because it lives in burrows.

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